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THE EFFECT OF WIND AND MOISTURE ON HEAT LOSS THROUGH THE FUR OF NEWBORN CARIBOU[1]

C. P. LENTZ AND J. S. HART

Abstract

The effects of air velocity and direction and of wetness on rate of heat transfer were studied in tests with samples of fur from five caribou calves. The rate of heat transfer through this fur increased by a factor of two to three with increase in air velocity from a negligible level (natural convection) to 23 m/second (direction parallel to sample). The effect of air velocity varied markedly (up to ±50% at 23 m/second) with inclination of the sample to the direction of air movement. Spraying water on the fur increased its rate of heat transfer markedly; addition of water equivalent to 10–12% of the volume of the fur doubled the rate of heat transfer. Other factors, such as erectness of the fur, direction of the hairs or "grain" of the fur with respect to the direction of air movement, and wetness of the skin also affected heat transfer. Differences between samples were large and did not appear to depend directly on the physical characteristics measured.

Introduction

Field studies have shown that wind, rain, and low temperature may be important environmental factors limiting survival of newborn caribou (5). As part of a study of the possible influence of these factors on survival of newborn caribou, the effect of wind, and to a lesser extent, of rain, on heat loss through samples of fur from newborn caribou calves has been studied. As will be shown, preliminary consideration of the mechanisms of heat transfer through fur and fibrous insulations, similar to each other in many respects, indicated the importance of wind and moisture, but there was little directly applicable information in the literature. Since no comparable investigation has previously been made, the results should also be of interest in determining the heat insulation qualities of other furs and of some fabrics.

Heat Transfer Review

The relative importance of each of the four mechanisms by which heat may ordinarily be transferred through fur (conduction, convection, radiation, and evaporation) may depend, in a given instance, on a number of interrelated factors including air velocity and direction, temperature, size and shape of

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sample, surface pattern or grain, physical characteristics (length, diameter, strength, density, and arrangement of hairs), and moisture content. Some indication of the effects of these factors on fur can be obtained from the comparatively abundant information available on insulations made of glass or organic fibers.

Conduction through air has been shown to be the main factor in heat conduction through glass fiber felts under conditions of interest in the present study (9). Other work with glass, flax, and other fibers showed that heat transfer with the fibers parallel to the direction of heat transfer was double that with the fibers perpendicular to the direction of heat flow (1). Radiation and natural convection were found to be minor in both investigations.

No work appears to have been done on the effect of air velocity on exposed fibrous insulations, although the effect of velocity on the air film heat transfer coefficient over various continuous surfaces has been studied extensively (6, p. 207). The air film coefficient is known to vary widely with direction of air flow (6, p. 216).

Moisture appears to affect heat conduction through insulation mainly by displacing air (4). Data on rigid insulations may not be applicable to fur, however, because of the difference in the nature of the "heat paths" produced by the water, and because fur may become "plastered down", decreasing its effective thickness. Since changes in relative humidity, within limits, have little effect on the amount of air displaced by water, relative humidity has little effect on heat transfer. Evaporation of water, on the other hand, may affect heat transfer through fur substantially, but no pertinent information is available.

Most previous work on fur was done to compare the heat-insulating quality of fur from different animals and to determine the effects of season and locality on insulating quality (2, 3, 7, 8). Most tests have been made with dry fur under still air conditions. Hammel (2) also measured the effect on insulating value of erecting or ruffling the fur (up to 50% increase), and of wet skin (5–20% decrease, presumably due to evaporation; it is doubtful if the skin itself contributes much to the insulating value of a thickly furred pelt). He found radiation to be a negligible factor. The effect of temperature corresponded to the change in the thermal conductivity of the air in the sample, as would be expected from insulation work (9). Moote (7) measured the insulating value of summer and winter samples of caribou fur over a range of velocities between 0 and 28 miles per hour (varied by a factor of two), with samples inclined at 45° to the direction of air movement.

This review indicates that air velocity and direction, and moisture content of the fur, have a large effect on heat transfer through fur, but present information is not adequate for quantitative prediction of the effect of these factors on heat transfer through the fur of newborn caribou. In addition, little is known about the relation between physical and thermal characteristics of fur. The effects of other factors such as temperature, radiation, and natural convection are either calculable or small.

Materials and Methods

Samples were taken from five pelts obtained from 1- to 3-day-old animals at Mosquito Lake, N.W.T., in June 1958. The pelts were salted and placed under snow for a few days before shipment in the frozen state to Ottawa where they were stored at −30° C. Seven samples (Table I gives physical characteristics) were tested, one from the back area of each pelt, and one from the side area of each of two pelts. No slipping of fur occurred in any of the pelts before or during the tests.

TABLE I

Description of samples of caribou fur tested
(All samples from animals 1–3 days old)

Sample	Fur depth combed down (mm)	Length of fur combed up (mm)		Hairs per cm²	Weight of fur (g/cm²)	Weight of animal (kg)	Remarks
		Guard hair	Under fur				
Calf 1 (male)							
Back	11	20	13	4300	0.020	5.1	Fine, soft, thin fur, relatively low proportion of guard hairs
Side	13	25	14	3900	0.018		Side has larger proportion of guard hairs than back
Calf 2 (female)							
Back	13	23	14	2600	0.022	5.6	Fur has larger proportion of guard hairs than calf 1
Calf 3 (female)							
Back	14	27	17	4700	0.033	6.0	Thick, relatively coarse fur, large proportion of guard hairs
Side	16	33	20	3800	0.030		Side similar to back but thinner
Calf 4 (female)							
Back	13	25	13	3000	0.019	5.1	Midway between calves 2 and 5 in softness. Relatively low proportion of guard hairs
Calf 5 (female)							
Back	12	25	13	5000	0.022	3.4	Fine, soft, thick fur, moderate proportion of guard hairs (similar to calf 2)

A 6-in. (15.2-cm) diameter hot plate (Fig. 1) centrally located in a 15-in. (38-cm) square wind tunnel was used for the tests. In most tests the hot plate was supported in a vertical plane on a vertical axis on which it could be turned for different inclinations to the direction of air movement. The hot plate was maintained at $38 \pm 0.2°$ C by liquid circulating through it while the room in which the tests were made was held at $1 \pm 1°$ C.

A 10.9 cm diameter heat flow disk* between the skin side of the sample and the surface of the hot plate was used to measure rate of heat flow (Fig. 1). The sample covered the entire hot plate to minimize the effect of edge loss. Samples were mounted on the hot plate with the skin wet, without wrinkles or looseness, but without stretching. Most tests were made with the fur combed down parallel to the direction of air movement, and care was taken to minimize error from variation in the erectness of the fur.

Measurements made included rate of heat flow, temperature difference through the sample, and air velocity in the tunnel. Measurements with insulation of known conductivity established the accuracy and reproducibility

*National Instrument Laboratories, 6108 Rhode Island Ave., Riverdale, Md., U.S.A. Catalogue No. HF-3.

of the heat flow disk as $\pm 2\%$ under the test conditions. The temperature difference through the sample was obtained from two fine wire (40 B & S gauge) butt-welded thermocouples in contact with the skin side of the sample (threaded under the surface membrane to ensure good contact) and a thermocouple in the tunnel air. The air velocity in the tunnel was measured with a vane anemometer with an estimated accuracy of $\pm 10\%$.

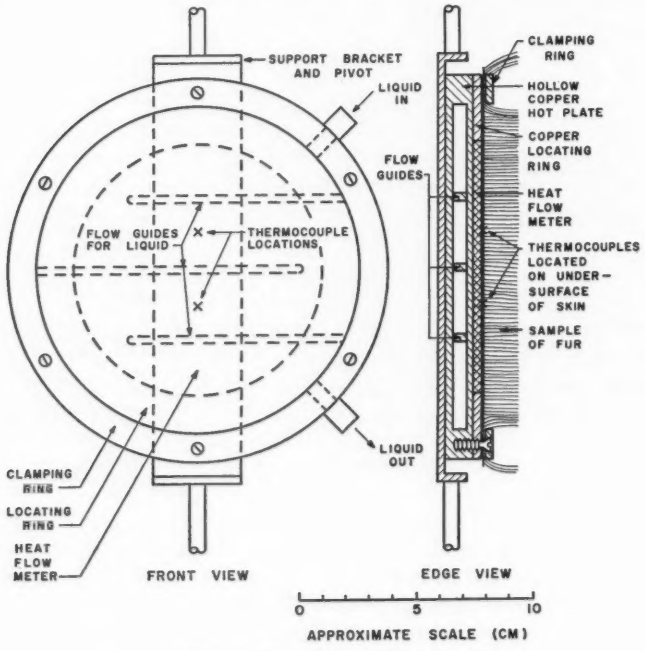

FIG. 1. Hot plate apparatus.

Each of the seven samples was initially arranged in line with the direction of air movement (nominally 0° angle, actually it was necessary to turn the sample toward the wind about 3–5° to obtain steady and reproducible measurements) and measurements made over a range of velocities from 0 (natural convection) to 23 m/second (51 miles/hour). The effect of inclination of the sample to the direction of air movement was determined at five velocities in this range for four of the samples by making measurements as the sample was rotated through 360° in 45° steps, at each velocity. In addition, the effect on heat transfer of other factors, including combing the fur erect, placing the sample with the direction of "grain" of the fur perpendicular to the direction of air movement, wetting the skin, and progressively wetting the fur by spraying it with water, were each measured for several of the samples. Care was required during the spraying of samples to obtain comparable results because the distribution of water in the sample and the tendency for the

surface to become "plastered down" (decreasing effective thickness, but perhaps also decreasing air movement through the fur) depended not only on the nature of the fur but on the vigor with which the atomizer was used.

The results are given in terms of heat transfer rate or conductance

$$\left\{\frac{\text{kilocalories}}{(\text{hour})(\text{square meter})(\text{degree C})}\right\}$$

rather than in terms of insulating value (clo units) because it was thought that rates of heat transfer would indicate more directly than insulation values the degree of stress to which animals are exposed by changes in thermal environment.

Results

Heat loss varied widely with both air velocity and direction, the effect of velocity depending on direction (Figs. 2, 3, and 4). The results in Figs. 2 and 4 indicate considerable variation between samples from different animals, in response to changes in both velocity and direction of air movement with respect to the sample. Although there was considerable difference in appearance between back and side samples from the same animal, there was little difference in performance, so that results were averaged for the two samples from

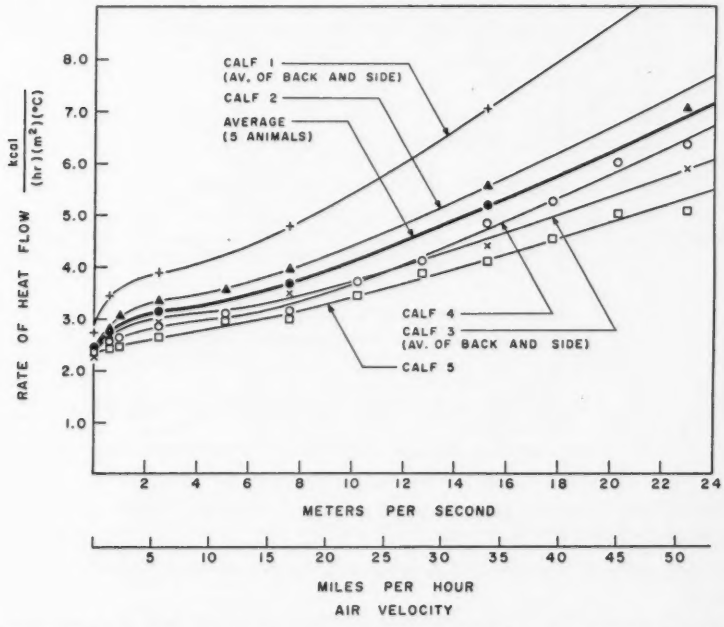

FIG. 2. Effect of air velocity on rate of heat transfer through the fur of newborn caribou with surface of samples parallel to direction of air movement. (Samples from backs of different animals except where otherwise noted.)

each of calves 1 and 3. The variation between samples from different animals increased with velocity, depending on direction, and was maximum at 90° (Fig. 4). Averages for all samples (Fig. 3) indicate that change in velocity had maximum effect at 90° and minimum effect at 225°. The results in Fig. 3 also show that the average of the rates of heat transfer at the different angles was approximately equal to the rate at the 0° angle. With the direction or "grain" of the fur perpendicular to the direction of air movement, the general shape of the curves was similar to those obtained with the direction of the fur parallel to the direction of air movement.

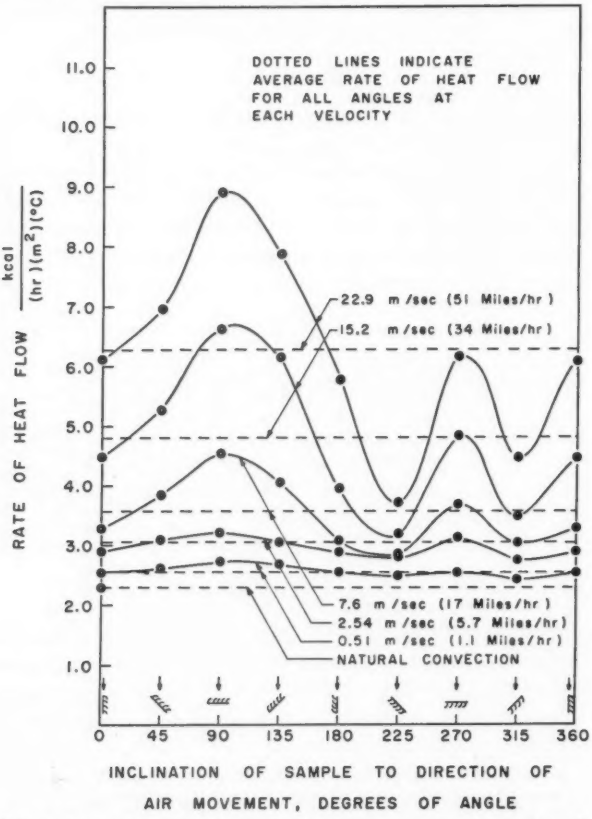

FIG. 3. Effect of sample inclination and air velocity on rate of heat transfer through the fur of newborn caribou, average of four samples from backs of different animals.

An indication of the effect of erectness of the fur on heat transfer is given in Table II.

Natural convection appeared to play little part in heat transfer through the fur since results obtained with the sample horizontal, fur up and fur down, and with the sample vertical did not differ appreciably.

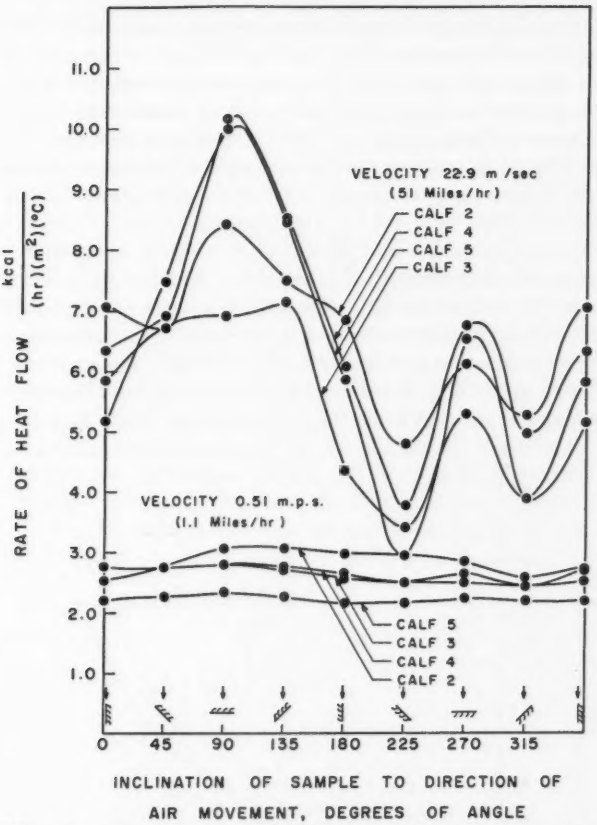

FIG. 4. Effect of sample inclination on rate of heat transfer through samples of caribou fur from the backs of different animals, at high and low air velocities.

TABLE II

Effect of erectness of fur on heat transfer
(Samples in line with direction of air movement)

Sample	Fur combed	Air velocity (m/sec)	Heat transfer, kcal / (hr)(m²)(°C)	Reduction in heat transfer (%)
Calf 1				
Back	Down	15.2	6.80	
	Erect	15.2	5.24	23
Side	Down	15.2	7.20	
	Erect	15.2	4.64	35
Back	Down	0	2.25	
	Erect	0	1.56	31
Calf 3				
Back	Down	15.2	4.35	
	Erect	15.2	2.64	39
Side	Down	15.2	4.40	
	Erect	15.2	3.47	21

Wetting the skin of the sample (before it was mounted on the hot plate) increased the rate of heat loss by as much as 50%, depending on the degree of wetness, at an air velocity of 15.2 m/second, but within 2 hours sufficient drying had occurred to reduce the rate of heat transfer to its normal level. Slight dampness of the skin did not affect rate of heat transfer. The insulating value of the dry skin was not determined directly but calculations based on measured thickness and estimated thermal conductivity indicate that it contributes little (less than 1%) to the insulating value of the pelt.

Spraying water on the fur increased heat transfer by up to 100%, depending on the amount of water added (Fig. 5). The effect per unit volume of water added was much greater for light and heavy wetting of the fur than for the intermediate range, namely, about 15% increase in heat transfer rate per 1% increase in water content in the 0–2% and 10–12% ranges of water content, compared with about 4% increase in heat transfer per 1% increase in water content in the 4–8% range of water content. The value quoted for cork and

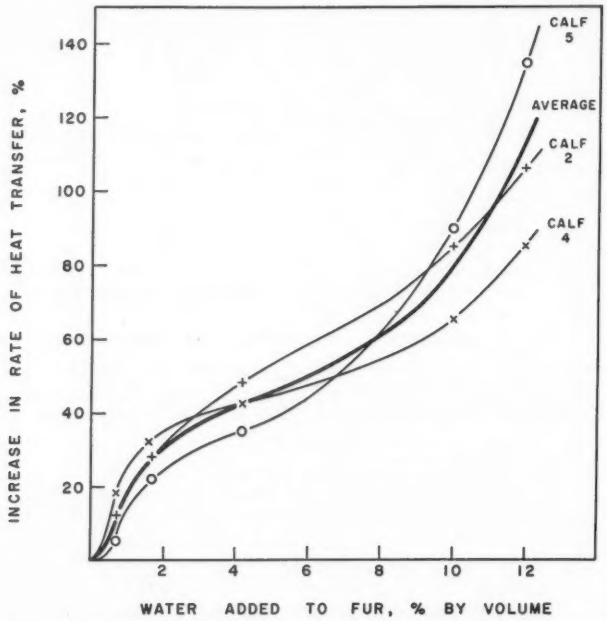

FIG. 5. Increase in heat transfer caused by water sprayed on fur. (All samples in line with direction of air movement, velocity 7.6 m/second for sample from calf 2, 15.2 m/second for samples from calves 4 and 5.)

NOTE: Visual effect of water sprayed on fur:

Water added (% by vol.)	Visual effect
0.7	Slightly wet, water in form of droplets in fur
1.7	Most water in form of droplets in fur
4.2	Some "plastering down" of fur on surface
10.0	Most of surface "plastered down"
12+	Fur wet through

glass wool is 6% increase in heat transfer rate per 1% increase in water content (4). The effect on fur may be partly explained by a large exposed surface area of water and consequently high rate of evaporation for low water content, by reduction in exposed surface area and in convection due to "plastering down" of the surface, for intermediate water content, and by water soaking through the fur with a consequently high rate of conduction, for high water content.

Discussion

The effect of velocity of air movement on the rate of heat transfer in these tests indicates that air movement does more than increase the air film coefficient of heat transfer. A number of other factors including flattening down and buffetting of the fur, and forced convection through the fur may also contribute to the increase in rate of heat transfer. The magnitude of this contribution is shown in Fig. 6 where the effect of air movement on heat transfer from a rough surface (6), on heat transfer through caribou fur (average of all samples) and through insulation of a fixed value equivalent to the caribou fur at negligible velocity are compared. At low air velocities the conductance of the caribou fur does not increase as fast as the conductance of the insulation, possibly because of flow conditions over the fur in this range of velocities.

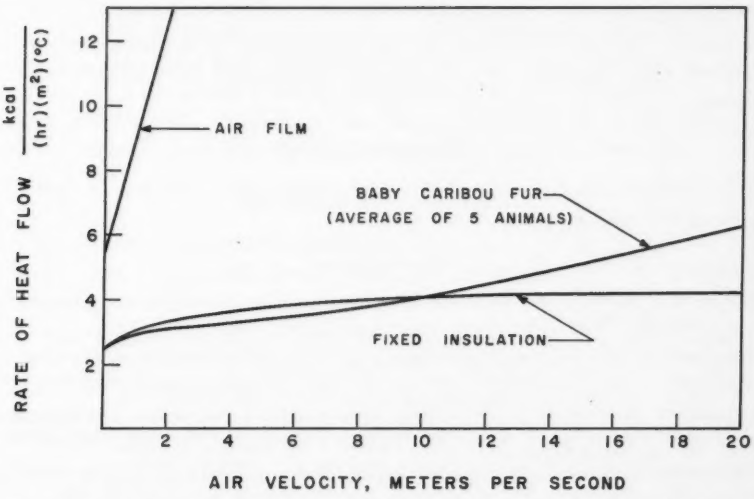

FIG. 6. Comparison of the effect of air velocity on the rate of heat transfer through the fur of newborn caribou, fixed insulation equivalent to the caribou fur at low air velocity, and through an air film only.

The following equations describe the three curves mathematically:

Air film:

$$K_1 = 5.33+3.68V, \qquad V<4.9$$

$$K_1 = 6.45V^{0.78}, \qquad 4.9<V<32.9$$

Fixed insulation:

$$K_2 = 4.50K_1/(K_1+4.50) \qquad (K_2 \text{ approaches } 4.50 \text{ as } K_1 \text{ becomes large})$$

Caribou fur:

$$K_3 = 3.0+0.026V^{1.60}$$

where K_1, K_2, K_3 = rate of heat transfer, kcal/{(hr)(m^2)(°C)}, V = air velocity, m/second.

No direct relation between the heat transfer characteristics of the samples and the physical characteristics listed in Table I, or any of the more obvious combinations of these characteristics such as length of fur and hairs per unit area or length of fur and weight per unit area, was apparent. This apparent lack of correlation indicates that more work will be required before thermal characteristics can be predicted from physical characteristics.

The results obtained in this study indicate that air velocity and direction, and wetness of the fur, affect heat loss through the fur of newborn caribou markedly. The combined effect of these factors may increase the rate of heat loss by a factor of four to six. It should be noted that these results give an approximate indication of the thermal stress to which a newborn caribou may be exposed but they do not give a quantitative estimate of rate of heat loss from the whole animal. Such an estimate would require a knowledge of the extent to which the factors governing heat transfer vary over the entire surface of an animal, as well as a knowledge of physiological factors which have not entered into this study.

Although this study has dealt mainly with the fur of newborn caribou, the results should be applicable in a qualitative way to some other furs and possibly to some fabrics.

Acknowledgment

The authors wish to acknowledge particularly the assistance of Mr. E. A. Rooke in making the tests.

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BLOOD-MEAL SOURCES AND NOTES ON HOST PREFERENCES OF SOME AEDES MOSQUITOES (DIPTERA:CULICIDAE)[1]

A. E. R. Downe

Abstract

Blood meals of engorged female *Aedes* mosquitoes collected from animal dwellings, light traps, and hand catches were identified by serological precipitin tests. The mosquitoes had fed on farm animals, humans, rodents, raccoons, deer, and probably foxes. Very few of the specimens had fed on birds and few contained the blood of more than one kind of host. Preliminary studies in a screened enclosure in a one-room barn that contained various combinations of domestic animals and humans indicated that a preference shown by mosquitoes for a mammalian host is related to the surface area of the host.

Introduction

Most *Aedes* mosquitoes found in the forest and woodland areas of Ontario and Quebec are avid blood-feeders and are generally acknowledged to be annoying and sometimes serious pests of man and domestic animals. Little precise information exists, however, concerning their host ranges and host preferences. Such information is necessary to assess the relative importance of various species as pests of man and animals and is of value in determining their roles as vectors of diseases.

This is a report on precipitin-test identifications of blood meals in *Aedes* mosquitoes collected from farm buildings, in light traps, and in insect net sweeps near farms and in wooded areas at Carleton Place, Ashton, Prospect, and Almonte in the Ottawa Valley. Collections were made in spring and summer from 1955 to 1957. Preliminary studies on host preferences of mosquitoes concerning man and certain domestic animals are also described.

Materials and Methods

Collection of Blood-engorged Mosquitoes

Window traps patterned after the designs of various authors (illustrated by Peterson (20)) were placed in barns and sheds housing cattle, horses, pigs, and chickens. Blood-engorged specimens were removed from the traps with an aspirator at least once a day, usually early in the morning. Blood-engorged specimens were also obtained with New Jersey light traps and a light trap described by McLintock (18). Each evening that the light traps were operated, a 6-lb block of dry ice wrapped in a cotton sack was hung on each of the traps as an added attractant. The traps were placed in fields and swamps about 100 to 150 yd from farm buildings and in relatively remote wooded swamps one-half to three-quarters of a mile from the nearest domestic-animal dwellings or pastures. Sweep-net collections of mosquitoes that were in flight or resting in grass and shrubbery also provided some blood-engorged specimens.

[1]Manuscript received December 24, 1959.
Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

Bird-baited traps were placed in wooded areas in an attempt to determine the attractiveness of avian hosts to *Aedes* mosquitoes. The trap was a modified mosquito-rearing cage (2 ft × 2 ft × 2 ft) with horizontal baffle entries on three sides midway between the top and bottom. Adult chickens or ducks were placed in the traps with their wings and legs tied securely with strips of cloth. The traps were either set on the ground or suspended from trees at a height of 6 to 10 ft.

For host-preference studies, a portion of a one-room barn was enclosed on three sides with fine-mesh mosquito screens that extended vertically from the ceiling to the floor, the fourth side being a wall of the barn. The enclosure measured about 17 ft by 10 ft and contained three stalls. The stalls were separated from each other by wooden partitions that were 5 ft high, and each stall was $5\frac{1}{2}$ ft wide by $6\frac{1}{2}$ ft long. In the barn-wall side a window was fitted with a trap. The vertical screen walls were fitted with horizontal ingress baffle entries (1, p. 291) about $4\frac{1}{2}$ ft from the floor. A horse, 2 calves, 2 humans, 1 pig, 3 dogs, and 12 chickens were placed in the stalls during various phases of the experiment.

Earlier studies (unpublished) had suggested that the preference of *Aedes* mosquitoes for a certain mammalian host was related to the surface area of the host. Animals and men were placed in the enclosure in a way intended to test this hypothesis (Table III). Early trials also showed that a given animal attracted about the same number of mosquitoes in each of the three stalls. Animals were placed in the enclosure about 4 o'clock in the afternoon and remained for at least 6 hours. The white-skinned, adult human males who took part in these studies wore light-cotton short-sleeved shirts, short trousers, shoes, and short socks. During one of the trials when a horse, a man, a pig, a dog, a calf, and six chickens were in the enclosure, a temporary partition (about 3 ft high) was built between the dog and the pig in one of the stalls. The chickens were restrained by tying their wings and legs and were, in most trials, placed on shelves built on the partitions between stalls at a height of $4\frac{1}{2}$ ft. All animals were weighed at least once during these studies. Surface areas were calculated with the equation $S = KW^{2/3}$, where S = area of surface, W = live weight of animal, and K is a constant depending upon the shape of the animal and the units of area and weight employed. K values for the animals used were given by Marshall and Halnan (17). Blood-engorged mosquitoes were removed from the enclosure with an aspirator early in the morning after each trial.

All mosquitoes collected were killed within a few hours with ether or chloroform and mounted on insect pins for subsequent identification. Dr. J. R. Vockeroth, Entomology Research Institute, Ottawa, provided generous assistance in the identification of many specimens.

Serological Methods

Sera used as antigens were collected from domestic animals in abattoirs and from nondomestic animals shot or trapped in the field. All sera were Seitz-filtered, bottled under aseptic conditions, and stored at −25° C until required for use.

Antisera were prepared by injecting healthy adult rabbits with the sera of humans, and domestic and nondomestic animals. Before injection, some of the sera were alum-precipitated after the method of Proom (21). Seven to 10 days after inoculation, each rabbit was trial-bled from the ear, and the antiserum tested for precipitin activity with the homologous antigen as described by Eligh (9). The titer was determined as the highest dilution of serum antigen that gave a positive precipitin-ring test with the constant dilution of antiserum. If titers from trial tests were sufficiently high (1:5000 or higher), larger quantities of blood were collected from rabbits by ear-bleeding or cardiac puncture. Antiserum was removed from the blood clot with the aid of centrifugation, Seitz-filtered, and stored at −25° C until required for use.

Group-specific antisera, similar to those described by Weitz (25), were prepared so that each mosquito extract to be tested was screened for likely hosts before specific reactions were performed. This meant that fewer tests with specific antisera were necessary and considerable material was conserved. The group antisera were prepared in rabbits by injecting mixtures of the sera of a particular group of animals so that the resulting antisera reacted with the serum antigens of each species of the group. The groups were as follows:

Group I: cattle, sheep, goat, deer.
Group II: human, horse, pig.
Group III: dog, cat, fox, raccoon.
Group IV: chicken, duck, pigeon.
Group V: squirrel, chipmunk, woodchuck, guinea pig.

The group IV antiserum was considered adequate for detecting all avian antigens. Tests showed that it reacted with loon, duck, goose, chicken, turkey, crow, pigeon, swallow, oriole, and sparrow sera.

Antisera for specific tests were prepared by injecting rabbits with the serum from a single animal, and the resulting antiserum was absorbed with heterologous antigens following the methods of Weitz (24, 25). For example, a mixture of sera of horse, beef, sheep, and pig was added to a predetermined amount of antihuman serum and refrigerated overnight. Cross-reacting antibodies for horse, beef, etc. were thus precipitated, leaving antibodies specific for human serum. Generally, these methods were satisfactory when suitable amounts of heterologous antigen in relation to antiserum were used. However, absorption of the antidog serum with fox serum reduced the homologous reaction (antidog serum with dog serum) to such an extent that the antiserum was no longer useful. This occurred also with antifox serum and dog serum so that specific tests were not obtained. No attempt was made to produce specific antisera for bird sera since few mosquito specimens gave positive reactions with group IV antiserum.

The blood meals were identified by methods previously described for testing black flies (7). The mosquitoes were removed from insect pins and crushed with sterile forceps in 5-ml beakers. One milliliter of 0.85% physiological saline (10) was added to each beaker and the insects were extracted for 4 hours at room temperature. Tests showed that longer extraction times were not necessary. The extracts were transferred in 0.05 ml amounts to

TABLE I

Numbers of mosquitoes of various species collected from domestic-animal dwellings that gave positive precipitin reactions for blood of various domestic animals and man* (Ottawa Valley, 1955)

Species	Site and dates of collection: Dairy barn: 13 cows, 1 bull, 4 calves (May 24–30)	One-room barn: 5 pigs, 1 cow, 2 calves (May 21–25)	One-room barn: 5 pigs, 1 cow, 2 calves, 12 chickens (June 10–19)	Section of two-room barn: 2 horses, 2 pigs, 4 calves (June 20–July 10)	One-room barn: 1 horse, 1 sow, 13 suckling pigs, 2 calves (June 7–20)	Totals
A. abserratus			2B	1B	2P, 2X	3B, 2P, 2X
A. canadensis					1H	1H
A communis	17B, 4X	11B, 14P, 3BP, 1X	10B, 13P	7B, 16H, 5P, 1HB, 2X	4B, 6H, 8P	49B, 22H, 40P, 3BP, 1HB, 7X
A. excrucians	16B	4B	23B, 16P, 1Hu	5B, 1H, 1X	14B, 11H, 19P	62B, 12H, 35P, 1Hu, 1X
A. implicatus	4B, 1X				4B, 1P	8B, 1P, 1X
A. intrudens	6B			7B, 6H, 2P	1H, 3P	13B, 7H, 5P
A. punctor	22B	19B, 16P, 1X		5B, 8H	6B, 5H, 11P	52B, 13H, 27P, 1X
A. sticticus	6B, 1Hu	1P	2B, 1P	4B, 2H, 2P	2B, 3H, 4P	14B, 5H, 8P, 1Hu
A. stimulans	68B	33B, 19P, 1X	41B, 33P, 1Hu	7B, 3H	26B, 19H, 45P, 1PHu	175B, 22H, 97P, 1Hu, 1PHu, 1X
A. trichurus	120B, 1Hu	53B, 41P, 1BP	74B, 59P, 1PF, 3X	64B, 62H, 10P, 1BH, 1X	42B, 37H, 83P, 2BP, 1HHu, 1HP	353B, 99H, 193P, 2BP, 1BH, 1HHu, 1HP, 1PF

*B, beef; F, bird; H, horse; Hu, human; P, pig; X, undetermined feeding.

small tubes (3 mm in diameter by 25 mm long) and antisera were layered beneath the extracts with Pasteur pipettes. Extracts of specimens collected in window traps, in light traps, or by sweeping were tested first with group-specific antisera and then with specific antisera. Extracts of specimens collected during host-preference studies were tested with specific antisera only. A positive reaction was indicated by the appearance of a precipitin ring at the interface between extract and antiserum. All antisera were dyed with safranine O as described by Herndon and Schubert (11) and negative (antiserum and saline) and positive (homologous serum and antiserum) controls were set up for each antiserum at the time of testing.

Results and Discussion

Mosquito Collections

The mosquitoes collected in greatest abundance were *Aedes trichurus* (Dyar), *A. stimulans* (Wlk.), *A. excrucians* (Wlk.), *A. punctor* (Kby.), and *A. communis* (DeG.). Species collected less frequently were *A. intrudens* Dyar, *A. implicatus* Vock., *A. sticticus* (Meig.), *A. abserratus* (F. and Y.). *A. trichurus* was the first species to appear in the spring and was usually abundant from the second week in May until the middle of July. Other species were present in greatest numbers from the end of May or early June until the end of July.

When favorable weather conditions prevailed, mosquitoes were collected from window traps in considerable numbers. Also many blood-engorged specimens, particularly of *A. trichurus*, were found resting on the rafters and walls of barns and sheds. It was estimated that only 1 of every 250 specimens collected in light traps was blood-engorged. The size of the catches in these traps was approximately tripled when dry ice was used as an attractant although the proportion of blood-engorged specimens in the catches remained about the same. A very low percentage of all mosquitoes collected by net-sweeping were blood-engorged, and many of these had probably fed on the collectors.

Generally, specimens that could not be specifically identified were not included in the tests. It is possible, however, that some of the specimens listed in Tables I–III as of *A. stimulans* were actually of *A. fitchii* (F. and Y.) as the adult females of these species are difficult to distinguish. Larvae of *A. fitchii* were found in pools with *A. stimulans*, *A. communis*, and *A. punctor* although they were not at all abundant. Similarly, some specimens identified as of *A. punctor* were possibly of *A. abserratus*, although larvae of the latter species were not found, suggesting that it is uncommon in the Ottawa Valley.

Host Blood Sources

The precipitin-test results (Tables I and II) for specimens collected in window traps, light traps, and net sweeps indicate the range of animals subject to attack by *Aedes* mosquitoes and also suggest the potential pest importance of all species to domestic animals. Observations on some of these

TABLE II

Numbers of mosquitoes of various species that gave positive precipitin reactions for domestic and nondomestic animals and man (1955) (the mosquitoes were collected by light traps and with insect nets)*

	Site and dates of collection						
	Grassy swamp, 150 yd from farm buildings. Horses, cows, and pigs housed in buildings (May 28–June 22)		Wooded swamp, ¾ mi from nearest farm animals or farm buildings (May 30–July 15)		Wooded swamp, ¾ mi from nearest farm animals or farm buildings (July 1–28)	Pasture, 4 goats, 2 calves, and several chickens present (June 9–17)	
Species	Light traps	Net sweeps	Light traps	Net sweeps	Light traps	Net sweeps	Totals
A. abserratus		1H				2B	1H, 2B
A. canadensis		1H, 1P		1H			2H, 1P
A. communis	6B, 1Hu, 1D, 3X	1B, 1P	6H, 4D, 1Dr	1H, 3Hu, 1HHu	5R, 2X	4G, 1D	7H, 7B, 4Hu, 1P, 6D, 4G, 1Dr, 5R, 1HHu, 5X
A. excrucians	1B, 4H, 1P, 2Hu	7H, 2P, 2X	3B, 3Dr, 1R	1Hu, 2R, 1F	1Dr, 1X	3B, 1G	11H, 7B, 3Hu, 3P, 1G, 4Dr, 3R, 1F, 3X
A. intrudens		5B	1D	1B		1G	6B, 1D, 1G
A. punctor	2H		1P, 2D, 4R	1Hu, 1X	1B, 1Rn, 2X	1B, 1Hu	2H, 2B, 2Hu, 1P, 2D, 1Rn, 3X
A. sticticus	2P		2Dr			1B, 1X	2B, 2P, 2Dr, 1X
A. stimulans	6H, 1Hu	3B, 1H, 2X	1P, 2R, 4Rn, 1F	9Hu, 1D, 1R	2Dr, 1G (or sheep) 3R, 3X	4B, 3G, 1Hu	7H, 7B, 11Hu, 1D, 4G, 2Dr, 6R, 4Rn, 1F, 3X
A. trichurus	11H, 2B, 2D	1H, 5P, 1DHu, 3X	3H, 3P, 5Dr, 1Rn	2Hu, 2R, 1DHu	3H, 2D, 2Rn, 4R	12B, 2G, 3Hu	18H, 14B, 5Hu, 8P, 4D, 2G, 5Dr, 6R, 3Rn, 2DHu, 3X

*B, beef; D, dog; Dr, deer; F, bird; G, goat; H, horse; Hu, human; P, pig; R, rodent; Rn, raccoon; X, undetermined feeding.

species attacking domestic animals have been made by various authors, and most of the abundant species are known to be pests of man. Although these results cannot be interpreted as indicative of host preference, it is noteworthy that a considerable number of specimens collected in areas rather distant from farm animals and their dwellings (Table II) had obtained blood meals from horses, cattle, and pigs. Also, specimens collected in the shed containing a sow and 13 suckling pigs showed a high proportion of pig feedings (Table I) when compared with the results from other collections.

Another feature of these results is that birds appear to be relatively unattractive hosts for the species studied. Natvig (19) has recorded attacks of *A. punctor*, *A. communis*, *A. excrucians*, and *A. intrudens* on nestling birds in Norway, but Hocking *et al.* (14) did not observe mosquitoes feeding on nestling birds at Fort Churchill, Manitoba. The traps baited with chickens used in the present studies attracted limited numbers of mosquitoes. After 23 evenings (summer of 1956) only four specimens of *A. punctor*, three of *A. intrudens*, three of *A. stimulans*, and one of *A. excrucians* had been attracted to two traps placed in a wooded swamp where mosquitoes were very abundant. Only three of these specimens (one of *A. punctor* and two of *A. stimulans*) had actually fed on the chicken. No mosquitoes were attracted to a trap baited with a domestic duck for five evenings. In contrast, a trap containing a white laboratory rabbit attracted an average of 19 mosquitoes an evening for nine evenings, and 91% of all specimens collected were blood-engorged. During the summer of 1957, some mosquitoes were collected in window traps in a chicken house near Marmora, Ontario. Most of the specimens were *Mansonia perturbans* Wlk., but nine specimens of *A. stimulans* and two of *A. excrucians* had fed on bird blood. Two specimens of *A. punctor* collected from the same window traps contained dog blood. It seems likely, therefore, that certain *Aedes* mosquitoes may feed on birds but probably prefer mammalian hosts. This point is illustrated further in Table III.

Few (0.87%) of the specimens (Tables I and II) contained the blood of more than one kind of host. It may be that intermittent feeding is rare among *Aedes* mosquitoes. Mosquitoes that were observed on horses and cattle appeared to be firmly attached to the host and were not readily disturbed once the feeding process had begun. These results are in contrast to those obtained by Rempel *et al.* (22), who reported that about one out of every three blood-engorged *Aedes* specimens they collected on the Canadian prairies and subjected to precipitin tests contained the blood of more than one kind of host. Specimens of *Aedes hexadontus* Dyar collected at Fort Churchill, Manitoba, had also fed on more than one host (6). The results from the present studies do not rule out the possibility of "multiple" feeding but, if it does occur, the first blood meal is probably in an advanced state of digestion (too far advanced to be determined by the precipitin test) before a second meal is taken. Other studies at the Ottawa laboratory showed that under field conditions most blood meals of *Aedes* specimens can be detected by the precipitin test 100 hours after feeding.

TABLE III

Numbers of mosquitoes of various species that gave positive precipitin reactions for domestic animals and man when animals and man were exposed to mosquitoes in a screened enclosure of a one-room barn (abbreviations as in Tables I and II)

	Combinations of animals in enclosure (surface area in sq. m in parentheses)						
	1 horse (5.05), 1 man (2.13), 1 dog (0.38), 6 chickens (0.79)	1 horse (5.05), 2 men (4.41), 1 dog (0.38), 6 chickens (0.79)	1 calf (1.91), 1 man (2.13), 1 dog (0.38), 6 chickens (0.79)	2 calves (3.43), 1 man (2.13), 1 dog (0.38), 12 chickens (1.35)	1 calf (2.02), 2 men (4.41), 1 dog (0.38), 6 chickens (0.73)	1 horse (5.05), 1 calf (1.91), 1 human (2.13), 1 dog (0.38), 1 pig (1.71), 6 chickens (0.73)	1 man (2. 13), 3 dogs (1.94), 12 chickens (1.25)
	No. 6-hr periods for each combination						
Species	4	4	4	4	4	7	4
A. communis	7H, 1Hu	14H, 12Hu, 1D	7B, 9Hu, 1BHu	4B	3B, 5Hu, 1DHu	6H, 5B, 1P, 3Hu	3Hu, 2D
A. excrucians	12H, 3Hu	8H, 11Hu	16B, 11Hu	20B, 6Hu, 1D	9B, 13Hu	8H, 2B, 3P, 2Hu	5Hu, 8D
A. intrudens	5H, 1D	3H	2Hu		2Hu, 1F	3H, 2B, 1P, 1Hu	
A. punctor	9H, 5Hu	6H, 4Hu	12B, 8Hu	7B, 3Hu	15B,* 17Hu	11H, 2B, 3P, 3Hu, 1D	4Hu, 9D
A. stimulans	26H, 4Hu	17H, 13Hu	19B, 21Hu, 1D	13B, 4Hu	16B, 30Hu	45H, 14B, 6P, 11Hu, 2D	9Hu, 13D
A. trichurus	23H, 12Hu	14H, 26Hu*	24B,* 16Hu	31B, 11Hu	17B, 22Hu	41H, 24B, 11P, 15Hu, 2D	12Hu, 5D
Total blood meals of all species	92H*	62H	78B*	75B*	60B*	114H	33Hu
	25Hu	66Hu	67Hu	24Hu	79Hu	49B	37D
	1D*	1D	1D*	1D*	1DHu	25P	
			1BHu		1F	34Hu	
			1F**			5D	

*i value greater than 2.
**i value greater than 3.

Most of the specimens giving negative reactions were probably collected when blood meals were in a state of digestion too far advanced to react with antisera. Also, other hosts may have been involved that could not be determined by the range of antisera used. A number of rabbits were seen in the areas where light traps were operated, and some unidentified blood meals were possibly of rabbit origin. It has already been mentioned that mosquitoes were attracted to a laboratory rabbit in a bait trap. An antirabbit serum was prepared in chickens, but extracts prepared from mosquitoes extracted with 0.85% physiological saline did not always give clear results when tested with this antiserum. Wolfe *et al.* (26) reported that more complete precipitation was obtained with antisera produced in chickens with 2% saline. Since blood-engorged mosquitoes were not collected from wooded areas in great abundance, it was considered inadvisable to extract specimens at a salinity unsuitable for general testing for the purpose of determining the presence of rabbit blood. No tests were made for the presence of amphibian or reptilian blood in any of the specimens. Many of the blood meals identified as dog blood (Table II) were probably of fox origin. Foxes were numerous in the wooded areas where light traps were operated, and there were a number of fox dens near the traps.

The results obtained with *A. communis* are of particular interest. Studies at Fort Churchill, Manitoba (2, 12, 13), indicated that this species did not take blood meals, and some evidence was presented that protein nitrogen requirements for egg development were obtained by flight-muscle autolysis in the female (12, 13). Vockeroth (23) has proposed that *A. communis* may be found to be nonbiting over the whole of its distributional range. Other authors (8, 15, 19) have reported this species attacking man, domestic animals, and birds. Hocking *et al.* (14) indicated that there were two "forms" of *A. communis*, separable from each other on the basis of proboscis-length/wing-length ratios. The "small form" (which exhibits flight-muscle autolysis) has a proboscis-length/wing-length ratio of about 0.74 while the "large form" has a ratio of about 0.85. The ratios of 50 specimens of *A. communis* collected in the present studies averaged 0.848 ± 0.025. Hocking *et al.* (14) suggested that the larger form was a separate species with habits similar to those of *A. punctor*.

Host Preferences

Results of the host-preference studies (Table III) indicate that, under the conditions of the experiments, apparent preferences of the mosquitoes for mammalian hosts are generally dependent upon the surface area of the host. When the surface area of one type of host was increased, the proportion of mosquito feedings on that host usually increased also. It cannot be concluded from these observations that the mosquitoes actually prefer larger mammals, since the fact that a considerable portion of field-collected specimens contained the blood of rodents, raccoons, foxes, and dogs (Table II) suggests that the latter group of animals are important as blood sources. It may be that *Aedes* species have no preferences for particular mammals but feed on the most readily available hosts in the field or, when there are two or more mammals close together, the mosquitoes land on larger animals by chance. Dow *et al.* (5)

reported that numbers of *Culex tarsalis* Coq. attracted to birds in revolving traps were proportional to the size of the birds. They also stated that birds of different species but of similar sizes attracted similar numbers of *C. tarsalis*.

For some of the species, the numbers collected were too small to attach much significance to the findings. When 15 or more specimens of one species were collected after trials with a particular combination of animals, the data were analyzed with a t test. Values for t were determined as follows:

$$t = (po - pt)/\sigma_p,$$

where po was the proportion of specimens observed to have fed on a particular host, pt was the theoretical proportion expected on the basis of surface area of the host, and σ_p was the standard deviation calculated from the theoretical or expected value. When t values were less than 2, the deviation of the observed proportion from the theoretical proportion was not considered to be significant. When values were greater than 2 but less than 3, the deviation was considered as suggestively significant, whereas t values greater than 3 were considered as indicative of a significant deviation (that is, factors other than surface area were influencing the results). Most t values for individual species were less than 2.

The t-test analysis assumed a normal distribution of mosquito feedings on all hosts. However, when the surface areas of chickens were included in the calculations, values for t tended to be higher than when surface areas of mammals alone were considered. The fact that no chickens were attacked by mosquitoes during many of the trials gave high t values for chicken-feeding. These results further indicate that the preference of most *Aedes* species studied is for mammals rather than birds.

The total blood meals identified in all mosquito species (Table III) attacking each combination of animals appear to be in general agreement with the postulated relationship of surface area of a given host and the number of mosquito bites the host receives. Occasionally these figures suggest that certain hosts are preferred, but such a conclusion might be misleading since there is no reason for assuming that the various species behave similarly with respect to feeding on or preferring the same host.

Statistical tests indicated that the blood-meal results were apparently not correlated with the weights of the animals. No attempts were made to regulate the diets of the animals nor were their respective colors considered. Khan (16) indicated that dietetic factors affected the attractiveness of white rats to *Aedes aegypti*, and other workers (3, 4) demonstrated the effects of different colors on mosquito attraction. The role of these factors in the determination of host preferences of mosquitoes are considerations for future study.

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A STUDY OF THE BLUE GROUSE (DENDRAGAPUS OBSCURUS (SAY)) ON VANCOUVER ISLAND, BRITISH COLUMBIA[1]

C. DAVID FOWLE[2]

Abstract

A study was made of the summer habitat, seasonal movements, fluctuations in populations, population density, breeding cycle, and summer feeding habits of *Dendragapus obscurus fuliginosus* (Ridgway). During the summer the grouse were found mainly in the early stages of the developing second-growth forests following fire or logging. The birds descended to the lowlands in late March and early April. Most of the adult males returned to the uplands by the end of July. The females and broods left the lowlands by the end of September. There is evidence of several well-marked fluctuations in the numbers of blue grouse in British Columbia since 1904. A density of about .2 adults per acre was recorded in late June and early July. Nesting took place in May and the first young appeared early in June. After the first of July it was not uncommon to see two females with their intermingled broods feeding together. The summer foods of both adults and young were almost entirely vegetable. Grit was the main item in gizzards from birds collected before the middle of July but later it was largely replaced by hard seeds. The grouse were not observed to drink free water except in captivity. There is a rough parallelism between frequency of occurrence of the main items of food in the environment and the proportion of each occurring in the diet.

This paper reports on a study of the blue grouse (*Dendragapus obscurus fuliginosus* (Ridgway)), a characteristic element in the fauna of young forest following logging or fire on Vancouver Island, British Columbia. In particular the summer habitat, seasonal movements, population density, reproduction, and summer feeding habits were investigated.

Field work was conducted on Vancouver Island in 1942, 1943, and 1944 at Cowichan Lake and particularly at Elk Falls and Quinsam Lake near the village of Campbell River. Supplementary observations were made on captive birds at the Campbell River Forest Nursery of the B. C. Forest Service. Laboratory work was carried out at the Department of Zoology at the University of British Columbia during the sessions 1942–43 and 1943–44 and at Campbell River.

Summer Habitat

The study area lies within Halliday's (7) Southern Coast Section of the Coastal Forest Region. Munro and Cowan (11) classify the area in the Coast Forest Biotic Area.

No detailed study was made of the vegetation at Cowichan Lake. The seral stages of the area are described by Cowan (5).

The study areas at Quinsam Lake and Elk Falls which originally supported large Douglas firs (*Pseudotsuga menziesii* (Mirb.) Franco) had been logged

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over a period of years until it was nearly denuded. The second growth was severely burned in 1938, resulting in a reduction in soil fertility and a retardation of regeneration. River bottoms, stream courses, and moister gullies supported a thick growth of alders (*Alnus rubra* Bong.), willows (*Salix* sp.), and some western red dogwood (*Cornus pubescens* Nutt). On higher ground, red-berried elder (*Sambucus pubens* Michx.) grew in thickets, often in company with ocean spray (*Holodiscus discolor* Pursh); Douglas fir, hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and cedar (*Thuja plicata* Donn) occurred in scattered associations. In general aspect, the area was almost free of trees (Fig. 1). On logged and burned areas there was in most sections an abundance of trailing blackberry (*Rubus vitifolius* C. & C.), cat's ear (*Hypochaeris radicata* L.), bracken (*Pteridium aquilinum* (L.) Kuhn), and a number of less-abundant species. A large area of the burn had been planted to Douglas fir seedlings by the B.C. Forest Service prior to 1943.

A plot survey was made at Quinsam Lake in 1943 to provide a rough estimate of the densities of the main items of ground cover on sections where grouse were abundant. Transects were run and at 50-pace intervals square-yard plots were measured off. The area shaded by each plant species or other type of cover was recorded for each plot. Any area less than one-half a square foot was tabulated as a "trace". Two areas (77 and 57 acres) totalling 134 acres were selected as typical of the study area around Quinsam Lake (Fig. 1). In Table I ground cover shading more than 1% of the total area of plots is tabulated by kind and by percentage frequency of occurrence. In all, 34 kinds of cover were recorded.

TABLE I

Percentage of area covered and frequency of occurrence of kinds of ground cover at Quinsam Lake, 1943

Kind of cover	Technical name	% covered		% frequency occurrence	
		Area 1*	Area 2*	Area 1	Area 2
Barren ground	—	19.0	26.0	85.0	71.0
Litter	—	9.0	8.5	68.0	71.0
Logs and stumps	—	13.5	12.0	20.0	17.0
Mosses and lichens	—	8.0	4.0	68.0	50.0
Bracken fern	*Pteridium aquilinum* (L.) Kuhn	2.0	3.0	20.0	17.0
Horsetails	*Equisetum* sp.	1.0	—	7.5	—
Grasses	Gramineae	.6	7.0	30.0	71.0
Willows	*Salix* sp.	1.0	1.0	30.0	19.0
Oregon grape	*Mahonia nervosa* Nutt. and *Mahonia aquifolium* Nutt.	.5	1.0	7.5	17.0
May leaves	*Achlys triphylla* (Smith) DC.	5.6	.4	45.0	17.0
Thimbleberry	*Rubus parviflorus* Nutt.	—	3.5	—	19.0
Trailing blackberry	*Rubus vitifolius* C. & S.	7.0	4.0	82.5	64.5
Willow herb	*Epilobium* sp.	1.0	2.0	65.5	52.0
Huckleberry	*Vaccinium parvifolium* Smith	1.0	.2	20.0	10.0
Salal	*Gaultheria shallon* Pursh	4.5	.4	50.0	10.0
Pearly everlasting	*Anaphalis margaritacea* (L.) B. & H.	3.0	.6	30.0	14.0
Thistles	*Carduus* sp.	.2	1.0	7.5	17.0
Cat's ear	*Hypochaeris radicata* L.	8.0	3.5	95.0	81.0

*Forty square-yard plots in area 1 and 48 plots in area 2.

PLATE I

FIG. 1. Portion of study area at Quinsam Lake.

In addition to plants listed in the table the following species shaded less than 1% of the plot area: liverworts, ferns other than bracken, sedges (Cyperaceae), Douglas fir seedlings, western red cedar seedlings, false Soloman's seal (*Smilacina* sp.), currant (*Ribes* sp.), violet (*Viola* sp.), bunchberry (*Cornus canadensis* L.), star flower (*Trientalis latifolia* Hook.), waxberry (*Symphoricarpos albus* (L.) Blake), red-berried elder, bluebell (*Campanula scouleri* Hook.), hawkweed (*Hieraceum* sp.).

It is, of course, impossible to say whether or not these samples were typical of the whole study area but they appeared to be representative of the part around Quinsam Lake at least. It is noteworthy that about 45% of the area of the sample plots supported no vegetation at all, being covered by logs, stumps, and litter or being quite barren.

Seasonal Movements

On Vancouver Island almost all blue grouse have moved up from the lowlands to the higher forested slopes by the end of September. They return to the lowlands in the latter part of March or the beginning of April. During this study, the young hatched early in June and shortly thereafter there was a noticeable decline in numbers of adult males. By mid-July it was difficult to find a male on the lowlands. On June 26 and 29 and July 2, 1943, males were still common, 30 out of 51 birds tallied being cocks, but after the middle of July few were seen. On August 13 and 14, 1944, 30 adults were tallied but only 1 was a male. The disappearance of males from the lowlands is illustrated in Fig. 2. Nearly all blue grouse had left the study area at Campbell River by September 15, 1943.

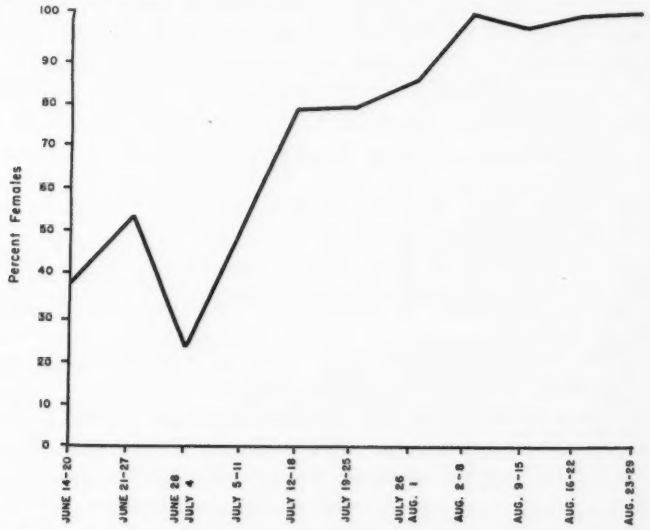

FIG. 2. Effect of emigration of males on percentage of females in total number of adult grouse observed in 1943.

The birds spend the winter roosting in coniferous trees and feeding upon needles (2, 15, 16).

The grouse return to the lowlands in late March and early April. Cowan (personal communication, 1944) stated that, in 1940, grouse at Cowichan Lake came down between March 20 and 23, and that hooting was heard at Goldstream Lake, Vancouver Island, after April 2. His notes record the first hooter on Gambier Island in Howe Sound, B. C., on March 28, 1943. On April 11–12, 1943, hooting birds were to be heard almost everywhere on the southern slopes of the Bald Mountain Game Reserve at Cowichan but few females were seen.

Population Fluctuations and Density

Leopold (8, p. 58), without presenting supporting data, states that "it is quite clear" that blue grouse populations are cyclic. While evidence for cycles is by no means clear, a study of the annual reports of the Provincial Game Warden (12), Game Commissioner (13), and Game Commission (14) reveals that since about 1904 several notable fluctuations in the blue grouse population in British Columbia have occurred. The trends described are based on reports of field officers and are not supported by quantitative data. Nevertheless, the striking contrast between reports covering periods of high and low population, and the general unanimity of the reports of the various officers, strongly suggest the occurrence of marked fluctuations.

In 1906, grouse in the interior declined to a low level (report for year 1917–1918) from a previous peak of population about 1903. There seems to have been an abundance of grouse in the province about 1912–13, followed by a decline to a low about 1919 and 1920. In 1915, grouse were very scarce in the interior. An "extraordinary disease in grouse of all kinds" reported at this time was said to be difficult to account for but may in some way have been correlated with "a regular plague of ticks". The Chief Game Guardian of Alberta reported that grouse in his province also declined at the same time. The 1916 report states that blue grouse in the interior of British Columbia were almost exterminated and, in spite of the fact that there was no hunting season in 1916 and 1917, the Provincial Game Warden had to report in 1919 that grouse were still scarce. Munro (10) reported blue grouse in the interior to be dying off owing to adverse weather conditions and to the presence of an "internal parasite".

The population gradually built up to a peak in 1924–25 and fell to a low in 1931. A high population was reported in 1935.

The inference to be drawn from Game Commission reports is that there was little change in number at the coast between 1935 and 1943. The interior birds seem to have declined somewhat to a low about 1937 and ascended again to a high level in 1941, or thereabouts. There is a suggestion of a general increase over the province to a peak about 1945, followed by a decline in the years following.

Population at Campbell River, 1943

Populations of grouse on four sample areas at Campbell River were estimated on June 26, 29, and July 2, 1943, and again on one of the areas on August 17, 1944. A strip-count census method was used in which one or two observers patrolled the areas at 1-chain intervals. All birds seen were counted, aged, and in the case of adults, sexed, and their position plotted on a map. The results of these censuses are presented in Table II.

TABLE II

Census data, Campbell River, 1943 and 1944

Area No.	Date	Area (acres)	Birds	Adults		Juv.	Acres per adult	Acres per bird	% adults
				M	F				
1	June 26/43	77	25	6	8	11	4.0	2.3	56
2	June 29/43	57	37	6	9	22	3.8	1.6	41
3	July 2/43	83	9	3	1	5	20.7	9.2	44
4	July 2/43	55	26	15	3	8	3.0	2.9	69
Totals and means (1943)		272	97	30	21	46	5.3	2.6	53
3	Aug. 17/44	83	62	0	14	48	5.7	1.3	23.5

It will be noted that density on area 3 is considerably less than on other areas. No reason for this could be found but it was noted that throughout the summer, birds were somewhat scarcer there than on surrounding areas. If the figure for area 3 is omitted an average density of one adult per 3.6 acres is obtained.

The figures suggest that at the end of June, 1943, about 50% of the population was composed of adults, there being about 2.2 young per female. In 1944, 37 females and 116 young were seen between August 13 and 17; the number of young per female was 3.1.

Reproduction

Hooting of males on the lowlands of Vancouver Island in late March and early April signals initiation of breeding. Hooting continues throughout May, and about the middle of June it may still be heard quite frequently through the day. Shortly after this, however, the number of hooting grouse may diminish somewhat until by the end of July only a few are heard. An exception was noted when two hooters were observed on August 13 and one was reported to be hooting on the same area on August 19–20. The cessation of hooting was due less to the silence of the birds than to their absence (Fig. 2).

Hooting may begin early in the morning and continue throughout the day and into the evening. At Campbell River, during June, hooters were noted to continue their calling until darkness fell about 9.30 p.m.

Observations from this study on territorial behavior and other phases of the breeding cycle are fragmentary but they fit in well with the pattern described by Bendell (2).

On Vancouver Island nest-building and egg-laying probably start during the first and second weeks in May (2). At Campbell River chicks were found for the first time on June 16. These were barely able to fly and were considered to be about 10 to 14 days old (4). Hence, allowing for an 18- to 21-day incubation (4) the eggs from which these birds hatched were laid some time during the second week in May. On June 29, a late nest was found containing four newly hatched chicks. It was a slight depression in the ground lined with grass and other dry vegetation placed under a log lying across a deer trail. Gabrielson and Jewett (6) record the nesting period of blue grouse in Oregon as extending from April 14 to June 4 and Bent (4) calculates the period derived from 60 records from Washington and Oregon as April 16 to July 12. Munro and Cowan (11) report nests of *fuliginosus* with eggs on May 20, May 24, and June 13. Three nests in the British Columbia Provincial Museum in Victoria bear the dates May 24, May 10, and May 7, containing five, six, and seven eggs respectively.

The hatch of the sooty grouse at Campbell River extended over several weeks. The first chicks appeared early in June and thereafter on into July. By the end of June, chicks of varying ages, some unable to fly and some strong on the wing, could be found. A late hatch was recorded on July 31 when a downy chick, scarcely able to fly, was found.

A count of 16 broods made between June 29 and July 2, 1943, yielded an average brood size of 2.9 with a range from 1 to 5. Wing, Beer, and Tidyman (17), working in Washington in the summer of 1940, record an average brood size of 4.25 for 12 broods at the end of June and beginning of July. From June 19 to August 22 they observed 99 broods for an average brood size of 3.65.

While the chicks were still small, broods were seen feeding separately. At this time it was common to see a female crouched on top of a stump while her brood fed on the ground below. If such a group was disturbed, the female often flew directly at the intruder and battered him with her wings. If she did not do this, she assumed an aggressive attitude, dragging her wings, hissing and cackling, all the while running from log to log or making short rushes at the intruder. Hens were particularly aggressive while the chicks were flightless or still weak on the wing, but were much less so when the chicks attained stronger powers of flight. At this time they often assumed the defensive attitude and uttered a few cackles before moving off quickly or flying away in the direction which the chicks had taken.

After the first week in July at Campbell River, two families were commonly seen feeding together, often with the two hens on adjacent stumps while the chicks fed around them on the ground. Frequently small, weakly flying birds were seen with others of almost twice the size and with greater powers of flight. On one occasion both hens were seen crouched head-to-tail on the same stump. There was no segregation of the chicks.

The adults were active in the evening until they went to roost just before dark. Very young chicks were probably brooded by females. Later, however,

flying chicks were found roosting in slash. In late August and early September, they were observed roosting in coveys of about a dozen birds. Once, birds were seen at twilight gliding in from all sides and settling down in the cover of a large area of cedar slash.

Summer Feeding Habits

Data on feeding habits were collected from analyses of crops and gizzards of grouse collected at Campbell River during the summer while supplementary information was derived from the examination of intestinal tracts, droppings, observations on captive birds, and field observations. Forty-five crops were removed on September 11 and 12, 1943, from grouse shot by hunters at Cowichan Lake.

Crops were preserved in a weak solution of formalin and examined in the laboratory. Volumes of foods were measured by displacement in water. All items occupying less than .2 cc in any crop were tallied as "traces".

Percentage volumes of food items are based on data from crop analyses only but percentage occurrence records are based on the combined results of crop, gizzard, and intestinal examination. The occurrence of an item in more than one organ in any bird was counted as a single occurrence. These data are presented in Tables III and IV.

Fleshy Fruits

Fleshy fruits, such as those of salal and trailing blackberry, comprised 57% of the volume of the sample, with most of these making their maximum contribution in the latter part of the summer (Table III). Fruits made their maximum contribution in September when 64% of the sample was composed of "berries".

A few differences in the diets of adults and young show up in Table IV. Salal was more highly favored by young than by adults while blackberries contributed a larger proportion to the adults' diet. During June, July, and August fleshy fruits comprised 32% of the adult diet and 47% of that of the young.

Green Foods

Green foods composed 41% of the total summer diet in the sample. Cat's ear contributed 25% and occurred in 70% of the birds examined. Buds, flowers, and leaves of this species made their largest contribution in August when they comprised 35% of the diet, with the adults consuming more than the young. In spring, cat's ear may have been the most abundant green food available on the burns. Stewart (15) reports that six crops collected in June and July in California, Oregon, and Washington contained 35.7% by volume of this item. Bracken was the second most popular green food consumed. In general, adults ate more green foods than the young birds.

Animal Foods

Animal matter made up about 2 to 3% of the summer food, being most abundant in the diet of young grouse which feed largely on animal matter

TABLE III

Food items, Campbell River and Cowichan Lake, 1942 and 1943 (% volume of foods on basis of 88 crops, adults and young; % occurrence of foods on basis of 89 birds, adults and young)

Item	No. birds					% total volume	% occur.
	2 (June)	10 (July)	23 (Aug.)	47 (Sept.)	6 (Oct.)		
Salal fruit (*Gaultheria shallon* Pursh)	—	4.0	25.0	58.0	17.0	45.0	61.0
Cat's ear buds (*Hypochaeris radicata* L.)	—	26.0	34.0	19.0	3.0	23.0	70.0
Bracken (*Pteridium aquilinum* (L.) Kuhn)	6.0	4.0	7.0	12.0	76.0	12.0	55.0
Blackberry fruit (*Rubus vitifolius* C. & S.)	5.0	22.0	14.0	—	—	6.0	32.0
Willow buds and leaves (*Salix* spp.)	77	—	5.0	—	—	2.0	5.0
Huckleberry fruit (*Vaccinium parvifolium* Smith)	—	1.0	—	3.0	—	2.0	11.0
Cat's ear leaves (*Hypochaeris radicata* L.)	—	—	4.0	1.0	—	2.0	23.0
Oregon grape fruit (*Mahonia* sp.)	—	—	3.0	1.0	—	2.0	8.0
Clover leaves (*Trifolium* sp.)	—	23.0	2.0	—	—	1.0	7.0
Blue bilberry fruit (*Vaccinium ovalifolium* Smith)	—	—	—	2.0	—	1.0	1.0
Waxberry fruit (*Symphoricarpos albus* (L.) Blake)	—	—	3.0	—	—	1.0	16.0
Twenty-seven other plants	12.0	18.0	2.0	2.0	3.0	2.0	—
Invertebrates	—	2.0	1.0	2.0	—	2.0	40.0

for the first 10 days or 2 weeks of life (1, 4). Mr. K. Racey of Vancouver, B. C., reported that on June 18, 1937, he collected a downy young male blue grouse at Alberni, Vancouver Island, and removed from its crop 13 green caterpillars, 3 snails in the shell, 1 spider, 1 ant, 1 sowbug, 21 leafhoppers, and 6 aphids. Only four downy young were examined at Campbell River but all contained invertebrates, mostly insects. Invertebrates occurred in only 2 of the 14 adults examined at Campbell river.

Grasshoppers (Acridiidae), ants (Formicidae), and sowbugs (Isopoda) were the most abundant items.

Grit

Grit averaged 3.6 cc and varied between 1 and 5 cc in the 20 gizzards where it composed all, or almost all, of the hard contents. In any one gizzard the grit was generally remarkably uniform in size, the average diameter being about 4 mm but varying from tiny grains of sand up to pebbles 7 mm in diameter. White or pale-colored pebbles predominated.

Gizzards collected in June contained grit as the dominant hard constituent but later in July, and until the first weeks in September, it was largely replaced by hard seeds. The total volume of hard material per gizzard remained at slightly less than 4 cc but in many cases pebbles were entirely lacking. The seeds of blackberry, bunchberry, waxberry, and rose were the ones most frequently used.

TABLE IV

Food items, Campbell River, 1943

(% volume of foods in 36 crops of 12 adults and 24 young, June, July, and August)

Item	Adults					Young				
	2 June	4 July	6 Aug.	% total volume	% occur.	0 June	6 July	18 Aug.	% volume	% occur.
Salal fruit	—	—	15	12	18	—	5	26	25	39
Cat's ear buds	—	55	47	42	65	—	12	32	31	64
Bracken fronds	6	7	14	12	47	—	3	5	5	42
Blackberry fruit	5	11	24	20	65	—	25	12	13	57
Willow leaves and buds	77	—	—	10	6	—	—	6	5	4
Cat's ear leaves	—	—	—	—	—	—	—	4	4	12
Oregon grape fruit	—	—	—	—	—	—	—	3	3	16
Clover	—	—	—	—	6	—	31	3	4	21
Huckleberry fruit	—	3	—	—	12	—	—	—	—	4
Waxberry fruit	—	—	—	—	—	—	—	4	4	36
Thimbleberry fruit (*Rubus parviflorus* Nutt.)		—	—	—	—	—	19	—	1	8
Miscellaneous ferns	—	12	—	1.0	12	—	—	—	—	4
Salal flowers	—	6	—	.5	6	—	—	—	—	—
Vetch (*Vicia* sp.)	12	—	—	1	6	—	—	—	—	—
Fourteen other plants	—	—	1	1	—	—	3	3	4	—
Invertebrates	—	5	—	1 (2 kinds)	18	—	1	2	1 (11 kinds)	50

Grit occurred in small quantities in 11% of the crops examined. One or two pebbles sometimes appeared in the intestinal contents or in the droppings.

Water

Bendire (3) and Marshall (9) say that blue grouse are often seen near water or that they drink frequently. Beer (1) says that the tracks of dusky grouse are frequently seen near springs and that hens may be seen leading their broods to water. During this study, however, grouse were never seen to drink in the field and captive birds were seen to take water on only three occasions. Free water may not be important during the season when berries and other succulent foods are available.

Feeding Behavior

Field observations and study of volumes of food in crops showed the grouse fed throughout the day. However, the largest volume of food was consumed in the evening, when feeding began about 6 o'clock. Crops from adults collected during this period contained up to 35 cc of food.

The young fed almost continuously throughout the day, usually confining their activities to a small radius within which they browsed greens and probably ate a few invertebrates. Cocks were frequently observed feeding and hooting alternately but hens, with broods, were never seen to feed during the day. Several females collected during the early afternoon had little or nothing in their crops. However, females were frequently seen foraging in the evening. During the periods of active feeding the birds moved about briskly, pecking at whatever food they could reach. When feeding on soft fruits on or near the ground they took several into the mouth in rapid succession and then raised their heads and swallowed. When feeding on green browse, such as clover or Douglas fir seedlings, the birds tilted their heads so that the longest length of the bill was employed in shearing off mouthfuls of foliage.

The volume of food taken at any one time varied considerably. The largest volumes were found in two crops from Cowichan which contained 50 and 57.5 cc respectively. A pair of captive adults on one occasion ate, in one day, 470 cc of soft fruits weighing 281 g. A captive male ate a mixture of 64 blackberries and raspberries in 10 minutes. A hen was observed to eat 20 blackberries in less than a minute. Some days later the cock ate 54 huckleberries (about 20 cc) in about 2 minutes.

Relation to Vegetation

In summer, blue grouse on Vancouver Island occur mainly in second-growth vegetation in areas where, as a result of logging and fire, thousands of acres have been converted from inhospitable habitat into productive areas. The early stages of succession provide important foods such as bracken, cat's ear, and fleshy fruits for the adults, and invertebrates for the chicks. The survey on the two sample areas at Quinsam Lake revealed that although about 45% of the surface of the plots was not covered by plants, there was enough vegetation to support a large population of grouse. The following

important grouse foods together covered about 20% of the area of the plots: bracken, willow, Oregon grape, trailing blackberry, huckleberry, salal, and cat's ear.

It is usually held that in the absence of strong preferences the volumes of various foods consumed will be more or less proportional to availability. In the case of the blue grouse, the degree of dispersion or, in other words, the frequency of occurrence of various foods at intervals over the bird's range should influence availability. Table V shows that there is a rough parallelism between frequency of occurrence of the six most available food plants on the plots and the proportion of each occurring in the diet. The adult diet seems to be fairly regularly related to the frequency of occurrences, but the high percentage of salal fruits in the diet of the young birds suggests some selection. The smaller volume of blackberry in the diet of the young is probably due to the fact that blackberries were abundant in July when most chicks were still feeding mainly on invertebrates.

TABLE V

Relation between availability of food plants and volume occurring in the diet at Campbell River, 1943

Plant	% frequency of occurrence on 88 plots	% volume in diet	
		Adult	Juvenile
Cat's ear buds, leaves	87.5	42	35
Blackberry fruit	73.0	20	13
Salal fruit	28.5	12.5	25
Willow leaves and buds	24	10	5
Bracken fronds	18	12	5
Oregon grape fruits	12.5	0	3

The extent of the influence of the birds on the vegetation is not known but in some areas it could be considerable. For example, salal at Campbell River bore only a moderate crop of fruit and was rather sparsely distributed, occurring in small clumps on about 28% of the plots. It is possible that nearly all the fruits produced were eaten by grouse and the seed scattered in the droppings of the birds. In the cases of such highly favored foods as cat's ear, the seed production of the species may have been considerably reduced owing to the removal of the fruiting portions by the grouse. In many places virtually all buds and flowers had been browsed.

In 1943 the grouse caused what appeared to be serious damage to Douglas fir seedlings. At Campbell River nearly all seedlings on the deforested area had been planted under the reforestation program of the British Columbia Forest Service but their survival seemed to be jeopardized by grouse browsing both newly planted and established stock. The most serious damage was done in the spring when the birds, having just arrived on the lowlands, removed many terminal and lateral buds as well as some needles from these trees. Recently planted seedlings standing in loose soil were occasionally uprooted. Repeated browsing resulted in greatly reduced annual growth. In the summer

of 1943 5-year-old trees which had been planted out 3 years before were found to be no taller than 2-year-old stock still in the nursey beds. The period of most serious damage lasted from the time the birds arrived in the spring until willow buds and other green browse began to appear in sufficient abundance to supply other food.

A recent report from the B. C. Forest Service (Mr. H. G. McWilliams, personal communication) states that although browsing appeared to be severe the seedlings were developing excellent root systems. Those missed by the grouse put out leaders a foot or more in length. A plantation set out in 1940 which was severely browsed in 1943 recovered a year or two later as more and more of the trees grew out of reach of the birds.

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INTERMEDIN AND CHANGE OF COLOR IN FROGS: A NEW HYPOTHESIS[1]

K. G. DAVEY[2]

Abstract

The indolalkylamines, tryptamine and serotonin, have a slight but distinct melanophorotropic effect on the melanocytes of frog skin. Other work has shown that the skin of amphibians contains large amounts of serotonin. The present work offers evidence that intermedin has an indirect effect on the melanocytes of frogs, that another compound, destroyed by monoamine oxidase and phenol oxidase, is involved in the normal darkening process, and that intermedin promotes the release of a pharmacologically active substance from frog skin in vitro. This is taken as evidence on which to erect the hypothesis that intermedin acts on the skin to release an indolalkylamine which then acts on the melanocytes to bring about the darkening of the skin.

Introduction

It is well known that the presence in the blood of intermedin (melanocyte-stimulating hormone), a secretion of the pituitary body, brings about the migration of the pigment in the melanocytes of frogs. In the absence of the secretion, the pigment remains contracted in the cell and the animal appears pale. The assumption that intermedin acts directly on the melanocytes has been implicit in most of the research in this field.

Embryologically, melanocytes are nerve cells, being derived from the neural crest tissues (10). It is not surprising, therefore, that ionic movements should play an important role in the migration of the pigment (7), nor that adrenalin should have a concentrating effect on the pigment (4). Intermedin, however, is a peptide (6), a class of compounds which is unlikely to have a direct effect on nerve cells.

Recently, the indolalkylamines, a class of compounds important in nerve physiology, have been demonstrated to have a slight but distinct melanophorotropic effect when injected into hypophysectomized frogs (2). Also important is the fact that the skin of amphibia contains large amounts of the indolalkylamine serotonin (5). In view of these facts, the following hypothesis has been formulated. Intermedin does not act directly on the melanocytes, but acts on elements in the skin, promoting the release of the indolalkylamine which is bound there. The presence of this indolalkylamine in a free state brings about the migration of the pigment. In the absence of intermedin, no indolalkylamine is released, any indolalkylamine free in the skin is metabolized, and the pigment returns to its contracted condition. This paper presents evidence in support of this hypothesis.

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Materials and Methods

The frogs used in this study were healthy adult specimens of the leopard frog, *Rana pipiens*. Hypophysectomy, when required, was accomplished by an oral approach, while the animal was under urethane anaesthesia. The progress of pigment migration was followed by photographing an area in the web of one hind foot at intervals after the injection of the material in question into the dorsal lymph space.

The isolated pieces of frog skin were prepared by removing the web from the foot of a frog and separating the upper and lower layers of skin. The two pieces of skin obtained in this way were pinned out on wax under a Ringer's solution devised by Novales (7). The pieces of skin were rinsed with this Ringer at 15-minute intervals until the pigment had contracted.

The intermedin used in these experiments consisted of fresh frog pituitary bodies ground up in a glass homogenizer using as the suspending medium Novales' Ringer (7) for use with isolated frog skin, normal frog Ringer for injection into intact frogs, or Yeager's Ringer (12) for use with the cockroach heart preparation. The lysergic acid diethylamide and bufotenin were obtained from the California Corporation for Biochemical Research, the iproniazid from the Hoffman-Laroche Company (Montreal), and the other chemicals from Nutritional Biochemicals Corporation.

The isolated heart of the cockroach, *Periplaneta americana*, is a preparation which has been used extensively in the pharmacology of insects (12); in the present study its sensitivity to the indolalkylamines has been used as a means of detecting the release of such chemicals from the skin of frogs.

Experiments and Results

Effect of the Indolalkylamines

When an indolalkylamine like tryptamine or 5-hydroxytryptamine (serotonin) is injected into the dorsal lymph space of a hypophysectomized frog, the pigment in the melanocytes expands slightly within 15 minutes and remains expanded for about thirty minutes. As was noted in a preliminary communication (2), the degree of expansion is not great and appears to be independent of dose. Figure 1 shows the same area in the web of the foot of a hypophysectomized frog before and after injection of serotonin.

These observations have been extended to include some compounds with a structure or pharmacological action similar to the indolalkylamines. Histamine, tyramine, and acetylcholine all share some pharmacological properties with the indolalkylamines, but have no melanophorotropic effect. On the other hand, tryptophane and 5-hydroxytryptophane, almost identical in structure with the indolalkylamines, were without effect on the melanocytes, indicating that the possession of an amine group is important. Further, the amine group apparently must be unsubstituted, because bufotenine (N,N-dimethyl serotonin), an indolalkylamine with a disubstituted amine group, failed to elicit a response from the melanocytes.

PLATE I

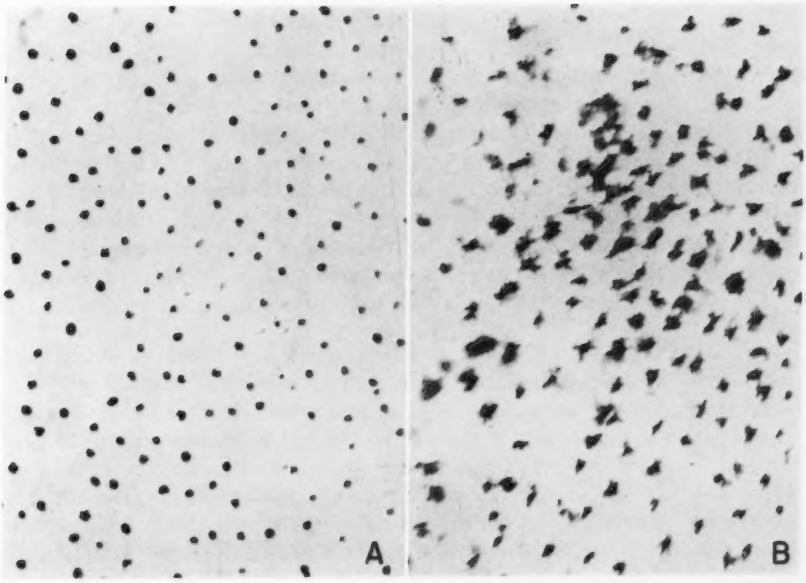

FIG. 1. Photographs of the melanocytes in the same area of a web of a frog's foot. A, before injection of 100 μg of serotonin; B, 15 minutes after injection. The frog had been hypophysectomized 2 days earlier.

All these experiments have been repeated using isolated skin. The results obtained with the intact animals were duplicated in these experiments.

In the preliminary report (2), it was stated that lysergic acid diethylamide (LSD), a compound which is known to inhibit many of the peripheral effects of serotonin (8), acted as an antagonist for intermedin. In the intact animal, injections of LSD along with intermedin were reported to result in a more rapid return of the pigment to the contracted state. A similar phenomenon has been reported for the toad *Xenopus laevis* (1).

However, further observations have shown that this effect of LSD is not invariable; in a few cases the return of the pigment to the contracted state is actually prolonged in the presence of LSD. In experiments using isolated skin, even very low concentrations of LSD brought about a complete migration of the pigment which lasted for some hours. Apparently, the action of LSD on the melanocytes of isolated skin is different from its action as antagonist to intermedin in the intact frog. In the latter case, it may be that the injection of LSD causes the release of adrenalin, which is already known to antagonize the action of intermedin (4). At any rate it is clear that the more direct action of LSD is to bring about a prolonged darkening of the skin.

The Indirect Action of Intermedin

It is well known that when intermedin, in the form of a suspension of pituitary glands in Ringer, is added to a piece of isolated skin, the pigment in the melanocytes expands, and remains expanded for a length of time dependent upon the concentration of intermedin in the Ringer bathing the skin. Eventually, however, the pigment returns to its contracted state. Now this may be a result of the inactivation of the intermedin or it may be a consequence of the exhaustion of some element in the skin which is necessary for the expansion of the pigment. The following experiment is an attempt to resolve this question.

Three identical dishes, each containing the same amount of Ringer were set up as follows. Dish A and dish B contained isolated pieces of skin pinned out on a layer of wax; the two pieces of skin were the upper and lower layers of the same web of a frog's foot. The pigment in each piece was completely contracted as the result of several rinsings with Ringer. Dish C contained no skin. At the beginning of the experiment dishes A and C received equal amounts of the same suspension of pituitary body.

The pigment in the melanocytes in dish A, of course, expanded, and then eventually contracted. When this had occurred, the Ringer in dish B was replaced with the Ringer from dish A, and dish A received the Ringer from dish C.

In each of the 10 trials, the pigment in the melanocytes of dish B expanded when the Ringer from dish A was introduced. The pigment in dish B remained expanded for a shorter time than in dish A, but in only two cases was this time less than 30 minutes. It is clear that the Ringer from dish A retains at least a part of its melanophorotropic activity, although this Ringer has ceased to have any effect on the melanocytes in dish A.

On the other hand, when the Ringer from dish C (containing intermedin which had not been exposed to skin) was added to dish A, the pigment in the melanocytes of dish A remained contracted (four cases) or exhibited only a slight and transient expansion lasting no more than 15 minutes (six cases). In those cases in which a slight expansion occurred, a third dose of the same concentration of intermedin failed to elicit a response. A higher concentration of intermedin, however, produced a dispersion of the pigment. The return of the pigment to the contracted state after a dose of the hormone is not, therefore, due to the decay of the hormone, nor is it a result of fatigue in the melanocytes themselves. Apparently some other element in the skin, necessary for darkening and controlled by intermedin, is exhausted.

The Involvement of the Indolalkylamines

If an indolalkylamine is involved in the normal darkening process, then the animal must possess some means of breaking down such a potent pharmacological agent. It should be possible to interfere with the processes leading to the destruction of the indolalkylamine and thereby potentiate the action of intermedin.

In searching for systems which might inactivate compounds like the indolalkylamines, attention is focused on two enzymes: monoamine oxidase, which is considered to be important in the destruction of serotonin, and the phenol oxidases, which would be important only if the phenyl portion of the indol ring possessed an OH group. Iproniazid is a widely used inhibitor of monoamine oxidase, and phenylthiourea inhibits the copper-containing phenol oxidases like tyrosinase. The following experiment demonstrates that the presence of these two inhibitors in the Ringer bathing a preparation of isolated frog skin potentiates the action of intermedin.

Two pieces of skin, the upper and lower layers respectively of a single web of the foot of a frog, were pinned out in separate dishes of Ringer. The pigment in the melanocytes of both pieces of skin was in the contracted state. To one of the preparations, phenylthiourea and iproniazid were added to achieve concentrations of 1.3 mg/ml and 0.5 mg/ml respectively. There was no change in the degree of dispersal of the pigment.

To each of the two skin preparations, enough of a suspension of pituitary glands was added to bring the concentration in each dish to an equivalent of one gland in 600 ml of Ringer. Previous experiments had shown this concentration to be below the threshold for pigment migration. In each of the six trials only the skin in the Ringer containing the inhibitors darkened; the pigment in the melanocytes of the skin without inhibitors remained contracted. It is apparent that when iproniazid and phenylthiourea are present together, the action of intermedin is potentiated. Experiments in which iproniazid or phenylthiourea were present alone did not give striking results.

The Release of a Pharmacologically Active Agent by Intermedin

If intermedin promotes the release of an indolalkylamine from the skin, it should be possible to detect the presence of this pharmacologically potent

material in the Ringer bathing a piece of skin which has been darkened by the action of intermedin. The following experiments were performed with this end in mind.

Two pieces of skin, each consisting of one-half of the skin from the dorsal surface of a frog, were rinsed with Ringer until the pigment in the melanocytes had contracted. These pieces of skin were placed in separate beakers, each containing 10 ml of Ringer. Beaker A contained in addition some intermedin, iproniazid (0.5 mg/ml), and phenylthiourea (1.3 mg/ml); beaker B contained only skin and Ringer. At the beginning of the experiment and at hourly intervals thereafter, aliquots of 0.5 ml were removed from each beaker and added to the Ringer bathing a cockroach heart preparation. The rate of beating of the heart was noted as the number of beats over a 1-minute period both before and 5 minutes after the addition of the aliquot. After each determination, the heart preparation was rinsed and no further determinations were made for at least 10 minutes. The effect of the addition of each aliquot is expressed as the percentage increase in heart rate. In this way, the appearance in the Ringer bathing the skin of a pharmacologically active agent could be detected.

In each of the six experiments performed in this way, the results were the same. The aliquots removed from beaker A (containing skin plus intermedin and inhibitors) increased the heart rate, the rate of increase, moreover, increasing with time. On the other hand, aliquots from beaker B increased the heart rate by only a small amount which did not increase with time. Significantly, the Ringer from beaker A increased the heart rate by a large amount only as long as the skin was dark; when the skin became pale again, the increase in heart rate returned to near its value at the beginning of the experiment. Table I gives the results of a typical experiment of this sort.

TABLE I

The effect of adding aliquots from two beakers to preparations of *Periplaneta* heart. Beaker A contains frog skin in 10 ml of Ringer, intermedin, iproniazid (0.5 mg/ml), and phenylthiourea (1.3 mg/ml); beaker B contains skin and Ringer only

Time in hours after adding intermedin	% increase in heart rate: aliquot from A	% increase in heart rate: aliquot from B
0	17	6
1 (skin dark)	33	9
2	41	11
3	50	10
7 (skin light)	16	10

Three experiments, identical with the preceding except for the omission of the inhibitors, gave the same qualitative results, although the percentage increase in heart rate achieved was not so great. Aliquots from control experiments in which the beakers contained no skin increased the heart rate by a small amount which remained uniform.

Discussion

The following hypothesis is under examination in this study. The presence of intermedin, a secretion of the pituitary body, is held to promote the release of an indolalkylamine from reservoirs in the skin. This indolalkylamine acts on the melanocytes, bringing about the migration of the pigment and the darkening of the skin. In the absence of intermedin, the indolalkylamine, which is continuously metabolized, disappears and the pigment returns to the contracted state.

Erspamer (5) has already demonstrated the presence of serotonin, an indolalkylamine, in the skin of some amphibia. Presumably, this highly potent material must be bound in some inactive form and must be protected from the action of enzymes. The present work has shown that the indolalkylamines tryptamine and 5-hydroxytryptamine (serotonin) have a melanophorotropic effect on the melanocytes of frogs. It is true, of course, that the degree of expansion of the pigment resulting from the action of the indolalkylamines is nothing like that encountered as a result of the secretion of intermedin. However, it is worth noting that a material from the reproductive tract of insects, believed to be an *o*-dihydroxyindolalkylamine, exerts a melanophorotropic effect which is somewhat greater than that resulting from serotonin (3). In this connection, it is important to realize that Erspamer's assay employed the oestrous uterus preparation. While this technique can achieve a considerable degree of specificity, it is uncertain whether it can separate serotonin from a dihydroxyindolalkylamine.

The action of LSD in bringing about a migration of the pigment in the melanocytes of isolated skin is at first sight difficult to reconcile with the hypothesis. However, the action of LSD on other peripheral systems is by no means clear. In many cases LSD is considered to antagonize the effects of serotonin (8), but in some instances it has been shown to mimic serotonin (4). In the present experiments there is the further complication that LSD may not act directly on the melanocytes but may release the indolalkylamine bound in the skin.

The hypothesis also requires that intermedin be shown to act indirectly. Isolated pieces of skin, after exposure to a particular concentration of intermedin, darken, but eventually blanch. That the blanching is not a result of the breakdown of intermedin is shown by the fact that the Ringer solution bathing the skin retains its melanophorotropic activity when placed on a fresh piece of skin. On the other hand, the blanched skin retains its ability to darken when a higher concentration of intermedin is present, indicating that the melanocytes themselves are not fatigued. Apparently other elements in the skin are exhausted by the continued presence of intermedin. According to the hypothesis, these elements are the reservoirs of indolalkylamine which are released by the action of intermedin. The fact that a higher concentration of intermedin promotes the darkening of a piece of skin already exhausted by a lower concentration can be explained by supposing that the indolalkylamine occurs in discrete elements, each of which has a different threshold for the release of the indolalkylamine by intermedin.

The experiments in which the action of iproniazid, an inhibitor of monoamine oxidase, combined with phenylthiourea, an inhibitor of the phenol oxidases, potentiates the action of intermedin show that these enzymes are probably involved in the breakdown of the substance which is responsible for the expansion of the pigment. Even if it is assumed that only one substance is involved, this evidence is not very meaningful. However, these experiments, taken together with the fact that the indolalkylamines alone among a large group of similar compounds can act as melanophorotropic agents, constitute indirect but convincing evidence that an indolalkylamine is involved in the normal darkening process. Moreover, the fact that the two inhibitors together are more effective than either one alone indicates that both monoamine oxidase and the polyphenol oxidases are capable of inactivating the active substance. If a phenol oxidase is involved, then at least one OH group must occur on the phenol portion of the indol ring. It is worth remembering that the end product of oxidation by the phenol oxidases is frequently melanin, and that prolonged darkening in frogs has been reported to lead to an increase in the amount of melanin to be found in the skin (9).

Finally it has been demonstrated that a pharmacologically active material is released by the action of intermedin on isolated frog skin. The cockroach heart preparation used in the assay of this material is sensitive to a wide range of compounds, but in view of the large amounts of indolalkylamine present in frog skin, it is likely that the pharmacologically active agent released by intermedin is this same indolalkylamine.

Acknowledgments

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BLACK FLIES (DIPTERA : SIMULIIDAE) OF IRRIGATION SYSTEMS IN SASKATCHEWAN AND ALBERTA[1]

F. J. H. Fredeen and J. A. Shemanchuk

Abstract

The immature stages of *Simulium vittatum* Zett., *venustum* Say, *meridionale* Riley, *tuberosum* Lund., *decorum* Walk., *bivittatum* Mall., *griseum* Coq., *arcticum* Mall., and *aureum* Fries, in decreasing order of abundance, occurred in 79% of 341 collections from 69 irrigation canals and 18 adjacent rivers and streams in irrigated areas of southern Saskatchewan and Alberta in the years 1950 to 1958. Canals less than 4 years old were infested despite a scarcity of aquatic vegetation which formed the main attachment sites. Factors reducing larval abundance included interruption of flow, lack of food and attachment sites, parasites, invertebrate and vertebrate predators, and possibly insecticides from irrigated crops. In some canals up to 100% of the larvae of *S. vittatum* were infected with nematodes, *Hydromermis* sp., which prevented pupation. Other larvae were infected with microsporidia. Most canals were dry in the winter. Larvae of only *S. vittatum* overwintered in two canals which flowed all winter; no viable overwintered eggs of any species were found in any canals. It is presumed that canals are reinfested each spring by gravid females immigrating from permanent breeding sites, mainly in the larger streams and rivers. Although black flies, especially *S. vittatum*, were occasionally abundant and all are blood feeders, they were generally less of a nuisance than mosquitoes. *Leucocytozoon* occasionally occurred in turkeys.

Introduction

Almost one million acres of farmland are presently irrigated in Saskatchewan and Alberta (Figs. 1 and 2) and at least another two million may eventually be irrigated. Black-fly larvae were discovered in some of the canals in 1950. This resulted in a study, ending in 1959, of the extent of larval infestations and of the economic importance of the adults. Attempts were made to relate larval abundance to various environmental factors and irrigation practices. In general, irrigated areas in the two provinces were found to differ considerably in physical features, in land use, and in other ways which apparently affected their suitability for the immature stages of black flies.

There is very little published information on black flies of irrigation systems. Townsend (13) warned that the immature stages of *Simulium meridionale* Riley (= *S. occidentale* Townsend) might occur in irrigation canals along the Rio Grande River. Edmunds (5) stated that *S. vittatum* Zett. and *S. griseum* Coq. occurred in irrigation drop structures in Nebraska.

Description of Irrigation Systems in Saskatchewan

Two-thirds of the 153,000 acres of irrigated land in Saskatchewan lie in the southwest corner of the province in the watersheds of the Cypress Hills (Fig. 1). The largest of these projects, all developed by the Prairie Farm Rehabilitation Administration, are at Val Marie, Maple Creek, Eastend, Swift Current, and Vidora and were completed in 1937, 1938, 1939, 1946, and

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1950 respectively. At Maple Creek there were some small private developments in operation prior to 1938. A number of other irrigation projects in south and southwest Saskatchewan are flooded only briefly during the spring runoff or for brief intermittent periods during the summer and are thus not capable of supporting black-fly populations.

The Cypress Hills irrigation systems are supplied with water from reservoirs on Maple Creek, Swift Current Creek, Battle Creek, Frenchman River, and a few smaller streams. Generally water flows continuously, only in the main canals, for about a five-month period from mid-May to mid-October. Most of the irrigated land is used for the production of forage hay crops; some of the land is in pasture and cereal crops.

Several small dairy herds, feed lots, and poultry establishments are located in these projects. However, livestock are not abundant until after the completion of the last hay cutting in the late summer when cattle and sheep are brought in from dry-land pastures to graze in the hay fields. Tractors rather

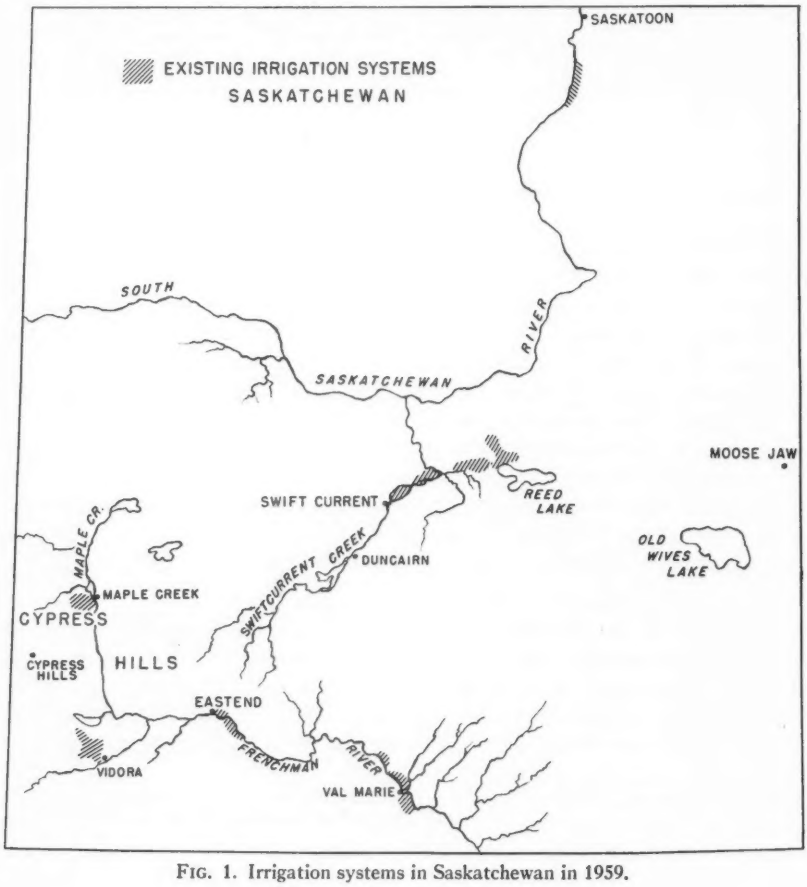


FIG. 1. Irrigation systems in Saskatchewan in 1959.

than horses are used in the farming operations. There are numerous farmsteads, several towns, and one city in or adjacent to these projects. Thus the black flies and other blood-sucking Diptera which develop in the irrigated areas are potentially as great a nuisance to man as to livestock.

The South Saskatchewan River Project, presently under construction, will provide for the irrigation of an additional 455,000 acres in an area north of the Cypress Hills projects.

Description of Irrigation Systems in Alberta

The construction of irrigation projects commenced in 1901 at Lethbridge and soon afterwards in several other areas in southern Alberta. In the early years, developments were sponsored by private companies and by the Canadian Pacific Railway. In the last 20 years, however, the Dominion and Provincial departments of Agriculture have been co-operatively responsible for all new developments. By 1958 about 820,000 acres were under irrigation (Fig. 2) and another 1,800,000 acres were proposed for irrigation. Most of the irrigated land lies in the area between Calgary, Cardston, and Medicine Hat, thus involving much of southern Alberta. Water is supplied from reservoirs on the Bow, Little Bow, Waterton. Belly, and St. Mary rivers; reserve water, partly from irrigation drainage, is held in a number of secondary reservoirs within the irrigation districts.

In general two sources of flowing water, both suitable for black-fly larvae, occur in each irrigation district. These include the supply water flowing from reservoirs in the "high-level" canals and laterals, and drainage water flowing in "low-level" canals or more often in natural streams. Permanently flowing water, suitable for overwintering larvae, occurs mainly in the several large tributaries of the Saskatchewan River originating in the Rocky Mountains, and rarely in streams originating in irrigation districts.

The various irrigation projects differ greatly in engineering and in agricultural development. The largest project, the St. Mary Irrigation District, is mainly well drained. In the oldest sections of this district, near Lethbridge, specialty crops such as canning and root vegetables predominate; in the newer sections cereal, oil, and forage crops predominate; in the newest sections, mainly near Medicine Hat where irrigation began in 1955, irrigation is confined mainly to pastures and to natural and cultivated hay crops. Generally irrigation is continuous in most areas from about the first week in May to the end of September, providing numerous excellent sites for black-fly larvae.

The eastern section of the Bow River District is well planned for proper drainage, and cereal, oil, and forage crops are grown. These require only intermittent irrigation, and black-fly breeding sites are rare. The Eastern Irrigation District has poor drainage facilities resulting in excessive amounts of waste water, which provide some excellent black-fly breeding sites in steadily flowing streams. Agricultural production there is confined mainly to forage crops and pasture for livestock. The Western Irrigation District and the foothills projects also specialize in forage and pasture crops which require continuous flow in main canals.

TABLE I

Range of physical and chemical conditions of water in irrigation canals and adjacent streams in Saskatchewan and Alberta in relation to the presence or absence of the immature stages of black flies, 1950 to 1958

Species	No. of coll.	Rate of flow (ft/sec)	Temp. (°F)	pH	Salinity (p.p.m.)	Suspended solids (p.p.m.)	Dissolved CO_2 (p.p.m.)	Dissolved O_2	
								Ml/liter	Saturation (%)
Simulium arcticum	12	4.0 or more	56 to 68	8.1 to 8.3	138 to 208	2 to 317	0	6.8 to 8.1	106 to 117
S. aureum	7	0.1 to 3.0	63 to 73	7.7 to 8.4	150 to 1070	14 to 443	0 to 3	6.5 to 9.7	101 to 160
S. bivittatum	22	2.0 to 4.3	55 to 76	7.6 to 8.7	120 to 290	14 to 720	0 to 4	6.7 to 9.6	108 to 143
S. decorum	28	0.8 to 4.0	45 to 80	7.3 to 8.9	148 to 730	2 to 104	0 to 3	6.5 to 9.8	100 to 152
S. griseum	22	1.4 to 4.0	55 to 77	7.7 to 8.7	165 to 512	8 to 720	0 to 3	6.9 to 9.6	108 to 143
S. meridionale	32	2.3 to 5.0	54 to 72	7.6 to 8.7	170 to 290	17 to 720	0 to 4	6.7 to 7.2	104 to 108
S. tuberosum	25	1.5 to 4.3	55 to 71	7.9 to 9.1	120 to 1820	2 to 720	0 to 1	6.8 to 9.6	106 to 143
S. venustum	50	0.5 to 5.0	52 to 74	7.3 to 9.4	162 to 740	2 to 443	0 to 4	6 to 9.8	100 to 143
S. vittatum	244	0.1 to 5.0	44 to 90	7.3 to 9.6	150 to 1920	0.1 to 720	0 to 12	4.8 to 11.7	67 to 190
No simuliids	67	0.1 to 5.0	44 to 88	7.3 to 9.4	144 to 2375	0.5 to 319	0 to 12	6.7 to 14	108 to 220

Blood-seeking black-fly adults should have little difficulty in locating a wide variety of donors in most of these districts except where cereal and oil crops predominate. Specialty crops require much hand labor, even though most agricultural operations are highly mechanized. Livestock populations are high, partly because of the great carrying capacity of irrigated pastures and partly because of numerous dairy, feed-lot, and poultry establishments, particularly in districts where by-products from specialty crops are available.

Methods

Potential black-fly breeding sites in most of the irrigation systems were visited only at irregular intervals from 1950 to 1957 in Saskatchewan and 1952 to 1957 in Alberta. However, during the 1958 irrigation season collections of larvae and pupae were taken at weekly intervals from 11 supply and drainage canals in five irrigation systems in Alberta and at monthly intervals from all other irrigation systems and from nearby rivers and streams. Of the total of 341 collections, 82% were taken in 1958. Collecting was done by hand without the aid of a quantitative sampler; approximately the same amount of time was allowed for collecting at each site. During analysis only the larvae and unemerged pupae were counted. Most collections were accompanied by physical, chemical, and biological data.

Water salinity was determined by measuring electrical conductivity (14), pH with a Beckman pH meter, and total suspended solids by weighing the dried residue filtered from a known volume of water. The dissolved carbon dioxide content was determined by titrating with $N/44$ sodium hydroxide, using phenolphthalein indicator (12), and the dissolved oxygen content by the Miller titration method (8). Oxygen values were converted to sea level equivalents and percentage saturation values using the conversion chart and nomogram of Rawson (9).

Black Flies

Black-fly larvae and pupae occurred in 79% of 341 collections from 69 irrigation and drainage canals and 18 adjacent rivers and streams. Nine species of black flies were collected but only *S. vittatum* occurred with any regularity (Table I). Physical and chemical data from infested and non-infested waters are shown in Table I and data on larval parasites in Table II.

TABLE II

The occurrence of black-fly larvae infected* with nematodes† and microsporidia‡ in irrigation systems in Saskatchewan and Alberta in 1958

	Sites with infected larvae (%)		Collections with infected larvae (%)		Maximum larval infection (%)	
Province	Nematodes	Microsporidia	Nematodes	Microsporidia	Nematodes	Microsporidia
Saskatchewan	23.8	66.7	16.7	47.7	70	95
Alberta	73.7	38.2	56.6	12.4	100	50

*Mainly larvae of *Simulium vittatum* Zett. infected.
†*Hydromermis* sp.
‡Probably *Nosema* sp.

Simulium aureum Fries

Larvae and pupae of this species were collected only seven times in the years 1950 to 1958 inclusive, and from five locations, all in Alberta. At all sites the pupae and larvae were scarce. Five of the collections were from three small drainage and supply ditches between Leavitt and Cardston in the Mountain View Irrigation District, one from a small drainage stream at Finnegan at the north end of the Eastern Irrigation District, and one from a drainage stream in the Bow River Irrigation District at Vauxhall. The larvae and pupae were found on pebbles and grass leaves usually in slowly flowing water (Table I). In 1958 this species was collected only in the period between July 12 and September 4.

Simulium vittatum Zett.

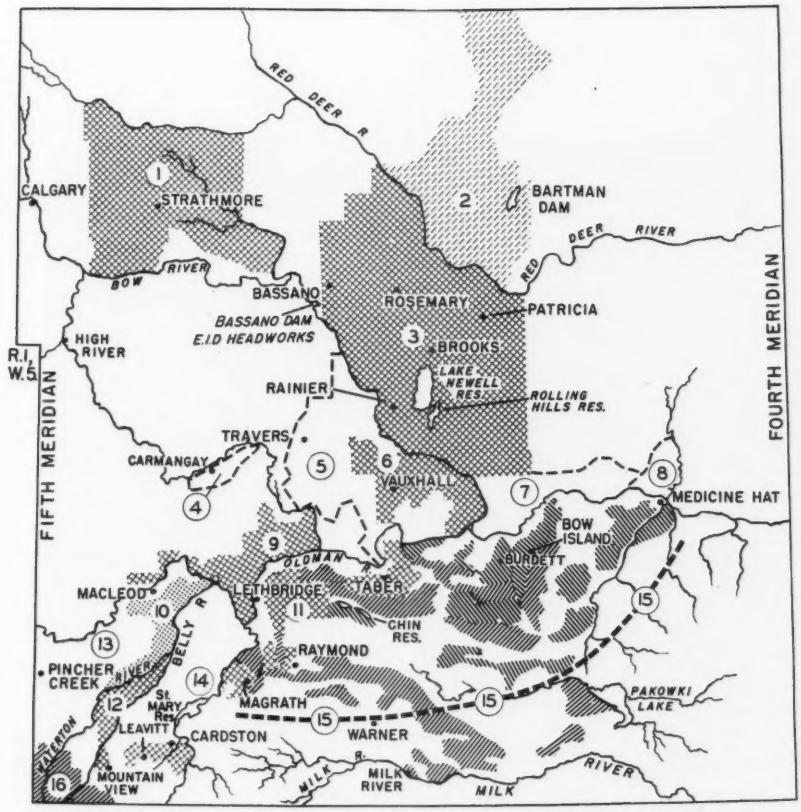
This was the most abundant and widely distributed species in all irrigation districts in Saskatchewan and Alberta. The only waters in which it did not occur were the headwaters of the five largest rivers in southwestern Alberta.

This species also had the widest distribution seasonally and larvae and pupae were found throughout the period April 17 (overwintered larvae) to September 30. The earliest and latest occurrences in 1958 in Alberta, in canals that had been dry the previous winter, were June 6 and September 30. This included practically the entire irrigation season that year since irrigation began about June 1 and ended in late September in most systems, although flow began in the main canals between May 6 and 14.

Overwintering larvae were found in a drainage stream and a supply canal at Vauxhall and Mountain View respectively. Although similar permanently flowing streams were rare, they apparently are the only sources of reinfestations each year. It is believed that adults emerging from these sites in the late spring oviposit in and thus repopulate the newly flowing canals each year.

Newly laid eggs were found in irrigation canals as early as June 30 in 1958. At some sites distinct increases in the numbers of pupae occurred around June 20 to 25, July 15 to 31, August 6 to 21, and September 3 to 11, indicating perhaps four complete generations in that irrigation season. Generally the larvae increased in abundance with each succeeding generation in Alberta, the usual occurrence where habitat conditions are satisfactory for the larvae throughout the summer. In many Saskatchewan sites the larvae decreased in abundance during the summer, indicating discontinuous water flow or some other deteriorating conditions.

Larvae and pupae occurred in all extremes of environment provided by irrigation (Figs. 3, 4, 5, and 6 and Table I), including flow as slow as 0.1 feet per second, and in most sites were more abundant than those of any other species. They were unusually abundant in certain canals at Val Marie, Eastend, Vidora, and Maple Creek in Saskatchewan and at Medicine Hat, Leavitt, Hays, Brooks, and Strathmore and especially at Taber, in Alberta. At the Taber site in 1958 (Fig. 3) water, fairly rich in plankton, flowed in a series of rapids at 5.0 f.p.s. down a rock-filled drop. Larvae and pupae often completely

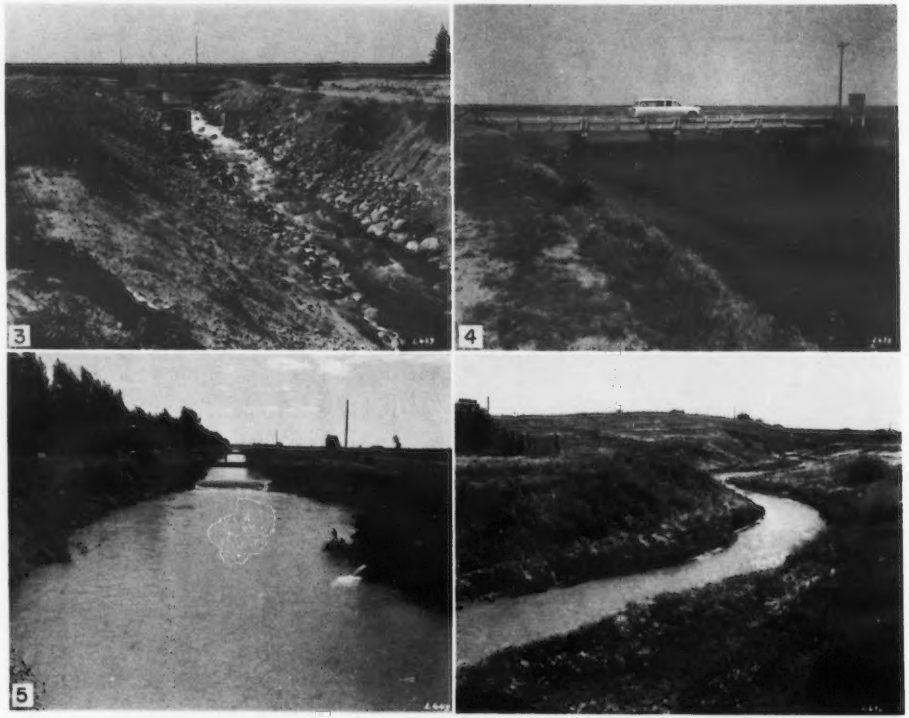


EXISTING AND PROPOSED IRRIGATION DISTRICTS – ALBERTA

1. WESTERN IRRIGATION DISTRICT
2. PROPOSED NORTH SASK. PROJECT
3. EASTERN IRRIGATION DISTRICT
4. PROPOSED CARMANGAY DISTRICT
5. PROPOSED RETLAW-LOMOND PROJECT
6. BOW RIVER PROJECT
7. PROPOSED REDCLIFF-RONALANE PROJECT
8. REDCLIFF-MEDICINE HAT EXTENSION
9. LETHBRIDGE NORTHERN IRRIGATION DISTRICT
10. MACLEOD IRRIGATION PROJECT
11. ST. MARY & MILK RIVER DISTRICT
12. UNITED IRRIGATION DISTRICT
13. PEIGAN I.R. 147
14. BLOOD I.R. 148
15. PROPOSED ST. MARY & MILK R. DEVELOPMENT
16. WATERTON LAKES PARK

FIG. 2. Existing and proposed irrigation systems in Alberta in 1959.

PLATE I



Fredeen and Shemanchuk—Can. J. Zool.

covered these rocks and any vegetation caught between the rocks. Water temperatures ranged from 44 to 79° F, pH 8.2 to 9.6, salinity 210 to 315 parts per million, suspended solids 8 to 35 p.p.m., dissolved CO_2 content nil, and dissolved O_2 content 8 to 9.9 ml per liter, 130 to 155% of saturation. There were few predaceous caddis larvae. Not more than 4% of the larvae were infected with microsporidia (probably *Nosema* sp.) and this was one of the few sites where none of the larvae was infected with nematodes.

It is believed that nematodes (*Hydromermis* sp.) were at least partly responsible for the scarcity of *S. vittatum* larvae in many sites which otherwise appeared to be quite suitable for larvae. Almost 74% of the Alberta sites and 24% of the Saskatchewan sites examined in 1958 contained nematode-infested *S. vittatum* larvae. In three of the Alberta sites 95 to 100% of the larvae at one time or another in 1958 were infested and at these sites few pupae occurred, indicating high larval mortalities.

Simulium meridionale Riley

This species was not collected from irrigation canals in Saskatchewan although it is known from rivers north of the irrigated areas. In Alberta, pupae were collected as early as June 6 (in 1958) and as late as August 19 (in 1953), from seven canals in the St. Mary Irrigation District near Lethbridge (Fig. 5), four in the Eastern Irrigation District near Patricia (Fig. 6), and one in the Western Irrigation District at Strathmore as well as the Bow River at Gleichen. Larvae and pupae occurred on grasses. They were never abundant at any one site but on three occasions were present in larger numbers than were those of any other species.

Simulium bivittatum Malloch

This species also was collected only from Alberta rivers and large irrigation canals. These were the Old Man, Bow, Little Bow, and St. Mary rivers and the Eastern, Western, and St. Mary Irrigation systems in the area between Cardston, Strathmore, Lethbridge (Fig. 5), and Patricia (Fig. 6). The earliest collection of larvae and pupae was on June 18 and the latest on September 4 both in 1958. On five occasions it was the most abundant of all species present.

FIG. 3. Water flowed at 5 f.p.s. in 1958 down this newly constructed drop in the Big Bend section of the St. Mary Irrigation District north of Taber, Alberta. The immature stages of *S. vittatum* were more abundant on these rocks than in any other irrigation canal investigated.

FIG. 4. The main canal at Hays, Alberta, in the Bow River District. Usually there were no black-fly larvae here in the summer of 1958; on two occasions a few larvae of *S. vittatum* were found. Scarcity of larvae was attributed to the sluggish flow of the 0.6 f.p.s. and to a lack of attachment sites.

FIG. 5. A main canal in the St. Mary Irrigation District at Lethbridge, Alberta. The immature stages of *S. vittatum, venustum, meridionale, bivittatum, griseum,* and occasionally *arcticum* were found at this site mainly on debris and branches of willows dipping into fast-flowing water near the drops.

FIG. 6. One Tree Creek, Patricia, Alberta, carrying drainage water from the Eastern Irrigation District. Prior to irrigation it was undoubtedly dry most of the time. Numerous attachment sites for larvae and pupae occur on rocks and on aquatic and emergent vegetation, and this stream is the main source of black flies in the region. *S. decorum, vittatum, venustum, bivittatum, meridionale,* and *tuberosum* were collected here throughout the entire summer of 1958.

These were on August 7, 1954, in the Spring Hill Canal at Rosemary; on July 16 and August 5, 1958, in One Tree Creek at Patricia (Fig. 6); and on July 16 and August 7, 1958, in the Bow Slope Canal near Rainier.

The larvae and pupae occurred on grasses and other aquatic vegetation.

Simulium griseum Coq.

With practically the same seasonal and geographical distribution as *S. bivittatum*, it too was confined entirely to Alberta. However, unlike that species, which was commonly found in rivers, *S. griseum* was found in only one small river, the Little Bow at Carmangay. Otherwise it was found only in irrigation canals and drainage streams in the St. Mary, Bow, Eastern, and Western Irrigation districts. Although often collected in combination with *S. bivittatum* it appeared to be more successful than *S. bivittatum* in colonizing grasses and other aquatic vegetation in irrigation systems and especially the slower-flowing water in the smaller ditches.

Simulium arcticum Malł.

Pupae of this species were collected as early as June 18 and as late as September 4 (both in 1958) in small numbers from vegetation and rocks in the larger, fast-flowing tributaries of the South Saskatchewan River such as the St. Mary, Highwood, Belly, Oldman, and Little Bow rivers and Lee Creek at Cardston, High River, Mountain View, and Brocket in Alberta. Very few pupae were collected from vegetation in large fast-flowing irrigation canals at Strathmore, Lethbridge (Fig. 5), and Brooks. Its rarity in irrigation canals indicated that these were only accidental extensions of its range. It was not collected from Saskatchewan irrigation systems although it was abundant in the Saskatchewan River north of the irrigated area. There it must be controlled annually to prevent damaging outbreaks.

Simulium decorum Walk.

This species was seldom abundant but pupae were collected with some regularity between June 18 and September 30 in 1958 on drop structures, head gates, rocks, and on vegetation down stream from these, in a few canals and small streams in the older irrigation systems in southern Saskatchewan and Alberta. In one location, a drainage stream at Patricia, Alberta (Fig. 6), its larvae and pupae were invariably more abundant than were those of other species throughout the entire 1958 season; in a similar drainage stream at Strathmore, Alberta, it was the most abundant species in early September of 1958.

Simulium tuberosum Lund.

Like *S. arcticum* this species was not collected in Saskatchewan irrigation systems nor in Alberta systems east of Taber and Brooks. However, pupae were collected June 6 to September 4 in 1958 from eight irrigation canals in five districts at Lethbridge (Fig. 5), Patricia (Fig. 6), and Strathmore. The very small numbers found indicated that these occurrences may have been accidental. Undoubtedly the sources of these infestations were the larger

tributaries of the South Saskatchewan River such as the Highwood, St. Mary, Little Bow, Oldman, and Belly rivers where *S. tuberosum* was regularly found although usually in small numbers. The pupae were generally attached to rocks and vegetation.

Simulium venustum Say

Although *S. venustum* was second in abundance and distribution only to *S. vittatum*, it was, however, much less abundant than that species. In the Saskatchewan irrigation districts it was not collected from canals but only from Frenchman River, Maple Creek, and Swift Current Creek, and these not after 1956. In Alberta the larvae and pupae occurred with increasing frequency in all irrigation districts west and northwest of Medicine Hat. In eight collections from canals at Patricia (Fig. 6), Strathmore, and Mountain View the larvae and pupae were more abundant than were those of *S. vittatum*. Other than in irrigation canals they occurred in the Bow and Little Bow rivers and Lee Creek.

The larvae and pupae were most abundant about the first week of June at Patricia, but not until mid-August at higher altitudes to the west at Mountain View. However, at Patricia they occurred throughout the entire period of June 6 to September 4 in 1958, indicating more than one generation. They were found attached mainly to aquatic grasses and other plants.

Factors Affecting Abundance of Larvae and Pupae

The development of irrigation systems has greatly extended potential breeding sites of black flies both seasonally and geographically in areas of Saskatchewan and Alberta that were previously too arid to produce streams except briefly during the spring thaw or during flash floods in the summer. However, the artificial regulation of water flow in irrigation systems imposes some restrictions upon the development of black flies. For instance, in some of the smaller systems, especially those in Saskatchewan, black-fly larvae and pupae were periodically eliminated during the summer of 1958 as a result of interruptions in water flow. It could not be proved that larvae of *S. vittatum*, or other species, could survive in pools of stagnant water as Edmunds (5) has suggested. However, with the resumption of flow in a canal, egg masses of *S. vittatum* soon appeared, apparently laid by females emerging from some nearby canal. Observations indicated that *S. vittatum* could complete its entire life cycle in less than 4 weeks. Other species of black flies were seldom found in those canals where the flow was interrupted during the summer.

In all but two systems in 1958 water flow ceased entirely at the end of the irrigation season in September and the canals remained dry until the following spring. The two canals which flowed all winter contained live overwintered larvae of *S. vittatum* in the spring of 1959. However, neither of these two canals nor four others that were dry during the winter contained overwintered eggs of any species. This indicates that most canals must be repopulated each spring by black flies emerging from nearby, permanently flowing rivers and streams.

Black-fly larvae generally are filter feeders, depending largely upon food particles carried to them by the flowing water. Thus the amount of food in the water and the rate of flow together undoubtedly affect survival and rate of growth of the larvae. The rate of flow in the canals generally ranged from 1 to 2 f.p.s. but less than this in some small canals and up to 5 f.p.s. in drops and flumes. Where the water was rich in plankton, as was the case from some secondary reservoirs, dense colonies of *S. vittatum* were found in flow as slow as 0.6 f.p.s. and smaller numbers of these larvae in water that was scarcely flowing at 0.1 f.p.s.

Populations of black-fly larvae were apparently limited in some canals because of a scarcity of attachment sites (Fig. 4). In canals less than about four years old, such as those in the Medicine Hat section of the St. Mary Irrigation District, there was very little aquatic vegetation, and larvae and pupae were scarce in 1958. In these canals larvae were found on timbers and cement drop structures and sometimes on emergent vegetation dipping into the slower-flowing water along the canal margins. However, in canals in the Eastern Irrigation District, aquatic vegetation (*Catabrosa aquatica* (L.) Beauv. (brook grass), *Potamogeton pectinatus* L., and *P. richardsonii* (Benn.) Rydb., *Equisetum* spp., and *Myriophyllum exalbescens* Fern.) often covered entire canal beds, providing excellent attachment sites for all species of larvae.

Excessive water turbidities apparently restricted the growth of aquatic vegetation and thus may have been indirectly responsible for the smallness of some populations of black-fly larvae. This was observed in the Lethbridge division of the St. Mary Irrigation District in 1954 when, as a result of construction of the St. Mary dam and new canals, the water carried up to 720 p.p.m. of suspended solids, and aquatic vegetation and larvae were scarce. At that time the water in certain canals in the Eastern Irrigation District contained less than 40 p.p.m. of suspended solids, and aquatic vegetation and larvae were relatively abundant.

The effects of other physical and chemical water conditions upon the abundance and growth of larvae were less clear. Water temperatures ranged from 44 to 90° F with variations as great as 29° F at one site in a single week. Temperatures of drainage waters ranged up to 16.5° F higher than those of supply waters. *S. arcticum* larvae occurred only in the cooler waters but *S. vittatum* larvae occurred at all temperatures (Table I).

Salinities ranged from 325 to 2375 p.p.m. in Saskatchewan and from 120 to 1920 p.p.m. in Alberta. Water in drainage systems invariably had higher salinities than that in supply systems. However, in most systems the salinities were highest in the spring, reached their lowest points around late August, and then gradually increased again. *S. vittatum* was the only species found in the more saline waters.

The pH of irrigation water ranged from 7.3 to 9.2 in Saskatchewan and from 7.3 to 9.6 in Alberta. Again only *S. vittatum* occurred in waters with the highest pH's.

The dissolved oxygen content ranged from 4.8 to 14 ml per liter. Saturation values ranged from 67 to 220%. The dissolved carbon dioxide content was nil in most locations but ranged up to 12 p.p.m. in others. Only *S. vittatum* larvae occurred in the extremes of these ranges.

Parasites and predators undoubtedly reduced populations of black-fly immatures in many localities. Microsporidial infections in larvae, apparently *Nosema* sp., were widespread in canals in Saskatchewan and Alberta (Table II). Only *S. vittatum* larvae were infected except at one site where *S. decorum* larvae were also infected.

Larvae infected with nematodes, *Hydromermis* sp., were also widespread, especially in Alberta. However, natural streams seldom contained infected larvae. Again, only *S. vittatum* larvae were infected except in one site at Mountain View where infected *S. venustum* larvae were also found. Nematode-infected larvae were more widespread in 1958 than in 1954 or in earlier years, and large networks of canals completed as recently as 1955 contained larval populations that were 100% infected with nematodes. However, in a few sites such as one at Taber, Alberta (Fig. 3), black-fly larvae were free of nematodes in 1958. Pupae were relatively abundant there the entire summer, indicating a high level of survival of the larvae.

Leeches (*Helobdella stagnallis* (L.), *Theromyzon occidentalis* (Verrill), and possibly *Mooreobdella fervida* (Verrill)) were collected from 7 of the 38 Alberta sites but not in Saskatchewan in 1958. *H. stagnallis*, the common pond leech, is known to feed on small invertebrates but it could not be shown that they fed on black-fly larvae. However, the leeches tentatively called *M. fervida* contained whole fifth- and sixth-instar larvae of *S. vittatum*.

Larvae of several unidentified species of caddis flies (Trichoptera) occurred in most irrigation canals and were often abundant; coleopterous and plecopterous larvae were less abundant. The extent of their predation was not investigated.

Small fish occurred in many canals. These included *Pimephales promelas* Raf., *Rhinichthys cataractae* (C. and V.), *Catostomus commersoni* (Lacepede), *Couesius plumbeus* (Agassiz), and *Hybognathus hankinsoni* Hubbs in decreasing order of abundance. Some of these, and especially *R. cataractae*, are known to feed on aquatic insects. Occasionally small unidentified fish were observed feeding on black-fly larvae, and damaged larvae were sometimes collected.

Insecticides applied to irrigated crops might affect black-fly larvae especially in canals receiving drainage water. A complete absence of larvae in the NE. Lateral system at Lethbridge throughout the summer of 1958 could not be attributed to anything else but insecticides. In earlier years this system was well populated with larvae. Nicholson (7) reported that 0.001 to 0.02 p.p.m. of DDT were recovered from waters of the Mississippi, Missouri, and Columbia rivers in the United States during a 3-month period in 1957. Several authors have stated that lesser dosages of DDT are toxic to black-fly larvae.

Economic Importance

It was not proved that black flies were of real economic importance in any of the irrigated areas. However, all nine species of black flies found developing in these systems are known to attack man, animals, or birds for blood. Farmers in the Eastern and St. Mary Irrigation districts reported outbreaks that caused considerable annoyance to man and animals.

Fallis and Bennett (6) showed that *S. aureum* and *venustum*, among others, fed naturally on ruffed grouse; infective sporozoites of *Leucocytozoon bonasae* Clark were found in *S. aureum*. Dr. R. Connell, formerly of the Canada Agriculture Health of Animals Branch at Lethbridge and now Director of the Veterinary Science Laboratory, University of Saskatchewan, reported (2) that turkeys infected with *Leucocytozoon* occasionally turned up at the Lethbridge Laboratory. Skidmore (11) demonstrated that *S. meridionale* (= *S. occidentale* Townsend) transmitted *L. smithi* (Lavern and Lucet) to turkeys in Nebraska, causing numerous fatalities. Edgar (4) reported an outbreak of *S. meridionale* affecting laying hens in South Carolina that reduced egg production from 70 to 20% in 8 days. *S. decorum* was induced to feed on domestic and wild birds by Davies and Peterson (3) when held in a vial over defeathered skin. It has been reported by several authors to feed on man.

S. vittatum, *bivittatum*, *griseum*, *arcticum*, *decorum*, *tuberosum*, and *venustum* have been reported by various authors to attack a variety of mammalian hosts. Outbreaks of *S. arcticum* have occasionally killed numerous animals in central Saskatchewan (1, 10) but authentic cases are not known from irrigated districts. A few livestock fatalities in the Eastern Irrigation District, attributed to black flies, were investigated in 1953. Some of the animals had died in April or early May, prior to the black-fly adult season, possibly as a result of eating poisonous range plants. Only one fatality occurred during the black-fly season but autopsy showed that this animal had died as a result of swallowing metallic objects.

Generally black flies were not as great a nuisance to man and animals as were mosquitoes. However, black flies may become more numerous in time, especially if aquatic vegetation becomes established in the new canals, providing additional attachment sites for the larvae and pupae. Canals of the 455,000-acre South Saskatchewan River Project, under construction in Central Saskatchewan, will undoubtedly become colonized by several of these species and perhaps others which already occur in natural streams in that area.

Acknowledgments

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ANOTHER HYMENOLEPID WITH GREAT MORPHOLOGICAL VARIATION, HYMENOLEPIS BENNETTI N. SP. (CESTODA) FROM NAPAEOZAPUS INSIGNIS ALGONQUINENSIS PRINCE[1]

REINO S. FREEMAN

Abstract

Hymenolepis bennetti n. sp. is described from *Napaeozapus insignis algonquinensis* Prince and *Peromyscus maniculatus gracilis* (LeConte) from Algonquin Park, Ontario. This cestode varies widely in the arrangement and number (one to eight) of testes, although 53% of the proglottides from seven worms were tritesticular and the majority of these had one testis poral and two antiporal. There were a number of abnormalities, mostly associated with unusual or incomplete segmentation of the proglottides. Anteroposterior reversal of proglottides also occurred. A number of Coleoptera, Orthoptera, Crustacea (Porcellionidae), and Mollusca, which ate cestode eggs, did not become infected.

Introduction

One to three stobilae of a new hymenolepid cestode occurred in 13 of 126 *Napaeozapus insignis algonquinensis* Prince and 1 of a larger number of *Peromyscus maniculatus gracilis* (LeConte) examined at the Wildlife Research Station in Algonquin Park, Ontario, during 1955 and 1956. This cestode is even more varied than the hymenolepids reported previously (4, 6, 9) so that a detailed study of the variations was made. This species might be included in any of three genera currently accepted by some workers in the subfamily Hymenolepidinae (10), although it does not completely fit any of them. Accordingly this species is placed in *Hymenolepis* Weinland, 1858 sensu lato and is named *Hymenolepis bennetti* n. sp. in honor of Dr. Gordon F. Bennett, who provided so many of the mice.

Hymenolepis bennetti n. sp.

(measurements in millimeters)

Hymenolepididae Railliet and Henry, 1909; Hymenolepidinae Fuhrmann, 1907; *Hymenolepis* Weinland, 1858. Strobila 59 (42 to 112)* in length, maximum width 1.79 (1.68 to 2.44) in postmature proglottides; 287 (278 to 391) proglottides all broader than long. Scolex 0.40 (0.27 to 0.39) in width with suckers 0.10 by 0.07 (0.10 by 0.08 to 0.13 by 0.10), rostellar ring 0.08 (0.07 to 0.12) in width, rostellum prominent with strong rostellar pad varying from globose to cone-shaped 0.11 by 0.08 (0.10 by 0.08 to 0.12 by 0.08) within rostellar sac 0.13 by 0.13 (0.14 by 0.13 to 0.16 by 0.14) (Fig. 2). Approximately 38 (36 to 40) hooks in single uneven row when rostellum retracted, but in even row when everted; of characteristic shape (Fig. 3), measuring 0.018 to 0.021 from tip of blade to tip of handle with prominent

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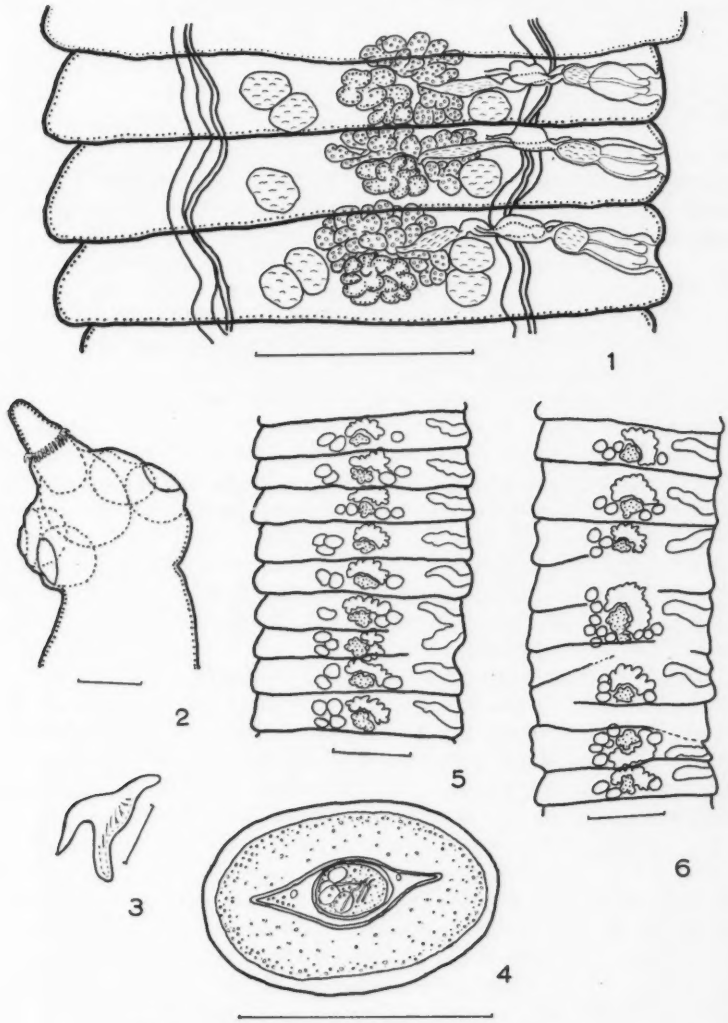
Contribution from Department of Parasitology, Ontario Research Foundation, Toronto, Ontario, Canada.
*Measurements in parentheses from six paratypes.

guard up to 0.017 in length. Minimum dimensions of neck approximately 0.31 (0.23 to 0.38). Genital primordia visible shortly after segmentation evident. A pair of larger ventral and smaller dorsal longitudinal excretory ducts throughout length of strobila; no transverse commissure. Sinistral, unilateral genital atria, near middle of margin of proglottis, with a plug in immature proglottides. Region of sexual maturity beginning with proglottis 210 (240 to 290).* Genital ducts pass dorsal to both excretory ducts. Cirrus sac 0.26 by 0.09 (0.20 by 0.08 to 0.36 by 0.08) (Fig. 1), with stout, short ductus ejaculatorius covered with numerous fine spines. Prominent internal and external seminal vesicles, usually sperm only in the internal one; no accessory sac. Vas deferens short and not coiled. Testes varying from 1 to 5 (1 to 8) in number, average 3.28 (3.05 to 4.27), median 3 (3 or 4); 1 poral and 2 antiporal the most common arrangement (35% of all proglottides examined), no uniform position (Figs. 3, 5, and 6); subspherical to elongate or ovoid from 0.09 by 0.08 to 0.13 by 0.10 (0.10 by 0.09 to 0.13 by 0.16) in maximum dimensions. Vagina, best seen in anterior part of strobila, opening into genital atrium ventral or ventroanterior to cirrus sac; terminal part beneath cirrus sac thick-walled and uniform in diameter; from 0.017 to 0.042 wide; remainder, thin-walled with numerous dilations, extending toward ovary and opening into fusiform seminal receptacle; sperm usually confined to latter. Ovary near middle of proglottis, highly lobulate, in some cases semidendritic, from 0.16 by 0.33 to 0.20 by 0.42 (0.20 by 0.23 to 0.23 by 0.48) (Fig. 1). Vitelline gland lobulate, but more compact than ovary, from 0.09 by 0.15 to 0.15 by 0.19 (0.10 by 0.13 to 0.16 by 0.26); position varies from somewhat lateral of ovary on antiporal side to directly medioposterior of ovary. Uterus begins as reticulum of ducts, and as proglottides mature uterus extends laterally well beyond excretory ducts, passing dorsally and ventrally to the latter, and occasionally between them; gravid uterus nearly fills proglottis. Reticulate nature of uterus obscure in gravid proglottides when egg-filled ducts are in intimate juxtaposition. Unfixed eggs ovoid, 0.053 by 0.035 to 0.069 by 0.044, with thick outer shell (Fig. 4). Space between shell and embryophore filled with fine granules. Embryophore 0.045 by 0.015 to 0.049 by 0.015 with attenuated ends which may be blunt, pointed (Fig. 4), or even slightly sinuous giving a slight "S" shape. Usually a large granule up to 0.003 and some smaller granules within the pointed ends of the embryophore. Embryo ovoid from 0.015 by 0.012 to 0.018 by 0.012, within ovoid cavity in embryophore. As embryo degenerates, as many as 15 cells become distinct. Six embryonic hooks; lateral pairs from 0.008 to 0.009, stout, with a distinct knob at juncture of blade and handle, blade strongly curved; median pair approximately 0.008, finer in structure, with less prominent knob, and blade not as strongly curved.

Hosts

Napaeozapus insignis algonquinensis Prince and *Peromyscus maniculatus gracilis* (LeConte).

*That proglottis in which uterus first visible, and usually where sperm first visible in vagina dilations.



(All drawings made with the aid of camera lucida.)

FIGS. 1–6. *Hymenolepis bennetti* n. sp.

1. Mature proglottides (scale 0.50 mm).
2. Scolex from paratype (scale 0.10 mm).
3. Rostellar hook from paratype (scale 0.01 mm).
4. Living egg (scale 0.05 mm).
5. Section of strobila from holotype (scale 0.50 mm).
6. Section of strobila from paratype (scale 0.50 mm).

Habitat

Small intestine.

Locality

Algonquin Park, Ontario.

Types

Holotype (39165) and four paratypes (39166) in U.S. National Museum Helminthological Collection; remaining paratypes at Ontario Research Foundation, Toronto, Ontario.

Comments

It is generally assumed that the number and arrangement of testes is stable among species in the subfamily Hymenolepidinae, particularly in those genera with three or fewer testes (3, 10). Therefore the variable number of testes (one to eight) in *bennetti* suggests that it might belong in *Oligorchis* Fuhrmann, 1906, which includes species with three to seven testes per proglottis, four or five being most common (3). Two paratypes of *bennetti*, if studied alone, doubtless would be placed in this genus. The new species differs from all others now in this genus by having nearly twice as many rostellar hooks and by the hooks differing in shape from all except *O. kwangensis* Southwell and Lake, 1939. Furthermore all the better-known species of *Oligorchis* are from birds (10), although the evidence is not conclusive that there is the high degree of host specificity among hymenolepids as proposed by Spassky (7, 8, and elsewhere).

H. bennetti n. sp. does not fit in *Rodentolepis* Spassky, 1954, since this genus normally has 8 to 10 rostellar hooks, apparently with a maximum of 12, although in other respects *bennetti* appears to belong here. The hooks are similar in size and shape with several species in this genus, e.g. *R. bahli* (Singh, 1958) and *R. johnsoni* (Schiller, 1952). Conversely, the hooks of *bennetti* differ markedly in shape from those of *Vampirolepis* Spassky, 1954, which are Y-shaped and have a comparatively long handle. This is one of the few hymenolepid genera, however, with as many as 50 hooks. Other than differing in hook number and shape, *Vampirolepis* and *Rodentolepis* appear synonymous. *H. bennetti* differs in one or more significant ways from all other hymenolepid genera from mammals listed by Spassky (8) and Yamaguti (10). Nevertheless, the creation of yet another genus for it appears unwarranted.

Several unarmed species now in *Hymenolepis* Weinland, 1858 sensu strictu have variations in testicular number somewhat comparable to those of *bennetti* (5, 6, 9, 10). Furthermore, a few armed hymenolepids from birds show similar, albeit less pronounced, variability (2, 4). This suggests that the number and arrangement of testes may not be as constant in this subfamily as was once supposed. Until the extent of variation becomes better known for those species which are armed it appears wisest to follow Czaplinski (1) and retain *Hymenolepis* sensu lato for those tritesticular hymenolepids which do not clearly fall into the few genera that are well delineated. Accordingly, the new species is Pescribed as *Hymenolepis bennetti*.

Variation in Hymenolepis bennetti

Variations in the number and arrangement of testes in *H. bennetti* are especially noticeable. The one to eight testes occurred in 24 different combinations in the seven worms from five hosts which were studied (Table I). Some of the more common combinations are shown in Figs. 5 and 6. The mean number of testes per proglottis per worm varied from 3.08 to 4.27 with an overall average of 3.52; the median number of testes per proglottis per worm was three in three worms and four in the remainder. Notice that there was nearly as wide a range among the three worms from a single host as there was in the worms from the remaining hosts (Table I). When observations on all seven worms are summarized, however, then 213 (53%) of the proglottides had three testes whereas only 122 (30%) had four testes, suggesting that *H. bennetti* is predominantly tritesticular. Possibly the few proglottides with only one or as many as seven or more testes resulted from a fusion of proglottides similar to that in Fig. 6.

TABLE I

Frequency of various combinations of testes in *H. bennetti* n. sp.

Hosts		*Napaeozapus insignis*						*Peromyscus maniculatus*	
Autopsy numbers		1447 (3 worms)			1450	1451	1807	1641	
		1	2	3					
Poral	Antiporal								
0	2	1	3		2				Total 6
0	3	4	4	1	2	2	4	2	19
0	4	1	1	1	2	1		1	7
0	5			1					1
1	0	1	1						2
1	1	1	1	2	2	3		2	11
1	2	28	13	5	25	15	37	17	140
1	3	5	9	13	8	11	2	14	62
1	4	3	2					1	6
1	5		2						2
2	0							1	1
2	1	9	4	4	12	5	14	4	52
2	2	4	8	7	7	15	2	8	51
2	3	2	5	5		6		2	20
2	4		3	3		1		3	10
2	5		1						1
3	0		1					1	2
3	1	1					1		2
3	2		1			1			2
3	3							3	3
3	5		1	1					2
4	1							1	1
4	2			1					1
4	3			1					1
Total proglottides examined		60	60	45	60	60	60	60	405
Median number of testes/proglottis		3	4	4	3	4	3	4	3
Mean number of testes/proglottis		3.28	3.87	4.27	3.22	3.68	3.08	3.77	3.52

The testicular pattern of one testis poral and two antiporal was the most common and occurred in 140 (35%) of all the proglottides examined (Table I). This was also the single most common pattern of distribution in five of the worms. The other common patterns were *1p 3ap* (15%), which was the most common pattern in one worm, *2p 1ap* (13%), and *2p 2ap* (13%), which occurred in one worm as frequently as did *1p 2ap*. The other 20 patterns of distribution were scattered among the remaining 24% of the proglottides.

Among the other variations which were noted, the most common was the unusual or incomplete segmentation of a proglottis (Table II) (Figs. 5 and 6). Frequently other abnormalities were correlated with this. In at least four cases there was a complete anteroposterior reversal of the proglottis (Fig. 6) where the vitelline gland was anterior to the ovary, and the testes were situated accordingly. In another case the ovary and vitellaria from two proglottides were fused and there were three poral and seven antiporal testes with only one set of genital ducts (Fig. 6). Another irregularity in four of the worms was the opening of two sets of genital ducts at a common genital pore (Table II) (Fig. 5). In other cases the proglottis was normal except that one or more of the organs such as the genital ducts, ovary, or vitellaria was missing or even more often abnormally developed. These variants occurred with no detectable regularity, and none of these variations were as common as those associated with the testes and referred to previously. The genital pores uniformly opened on the right lateral margin in five of the seven worms. In the specimen from *Peromyscus maniculatus* the only abnormality observed was that one or more of the proglottides had a sinistral pore in four sections of the strobila.

The living egg varied in size and in the shape of the hyaline embryophore, but no count of these variations was made.

TABLE II

Frequency of various abnormalities in *H. bennetti* n. sp.

Hosts	*Napaeozapus insignis*						*Peromyscus maniculatus*	
Autopsy numbers	1447 (3 worms)			1450	1451	1804	1641	
	1	2	3					
Unusual or incomplete segmentation	8	4	*	3	*	1		Total 16
Irregular alternation of genital pore					1		4	5
Two sets of genital ducts to common pore	3	1	3	1				8
Genital ducts missing		5			1			6
Ovary missing, misplaced, or malformed		2	2		2			6
Vitellaria missing, misplaced, or malformed		2	2					4
Total	11	14	7	4	4	1	4	45

*Difficult to tell because specimen stretched.

Life History

Eggs of *H. bennetti* were fed to the following terrestrial invertebrates to try to discover the intermediate host, but no infections were established.

Coleoptera: Seven adult Scarabeidae, four adult and four larval Silphidae, six adult Carabidae, and seven adult Tenebrionidae.

Orthoptera: Two adult and four nymphal Gryllidae and three adult Locustidae.

Crustacea: Eight Porcellionidae.

Mollusca: Fifteen terrestrial snails, mostly *Zonitoides arboreus*.

Acknowledgments

I thank Dr. A. M. Fallis, Director, Department of Parasitology, Ontario Research Foundation, for his support and suggestions, as well as the many people who provided mice, the Ontario Department of Lands and Forests for field laboratory facilities, and the Province of Ontario for a research grant from the Department of Planning and Development.

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NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

VI. AMPLICAECUM CACOPI, THELANDROS SP., RHABDIAS RANAE, AND OSWALDOCRUZIA MELANOSTICTI SP. NOV., FROM FROGS[1]

S. P. GUPTA[2]

Abstract

Amplicaecum cacopi Chatterji, 1936, *Thelandros* sp., and *Rhabdias ranae* Walton, 1929 are recorded, and *Oswaldocruzia melanosticti* sp. nov. is described, from frogs in East Pakistan.

Heterocheilidae Railliet and Henry, 1915

Amplicaecum cacopi Chatterji, 1936 (Figs. 1–5)

Material: Two males, one female.
Host: *Rana tigrina*.
Location: Small intestine.

Description

Worms of medium size. Cuticle smooth with fine transverse striations. Mouth (Fig. 1) surrounded by three well-developed rectangular lips, one dorsal, two subventral, all with dentigerous ridges of small teeth on the inner margins. Interlabia present. Dorsal lip bears two large papillae. Each ventrolateral lip with one large papilla towards ventral side and one very small papilla laterally. Oesophagus long. No ventriculus, no distinct bulb. Single intestinal caecum (Fig. 2), narrow anterior, broadest at union with intestine. Excretory pore immediately behind nerve ring.

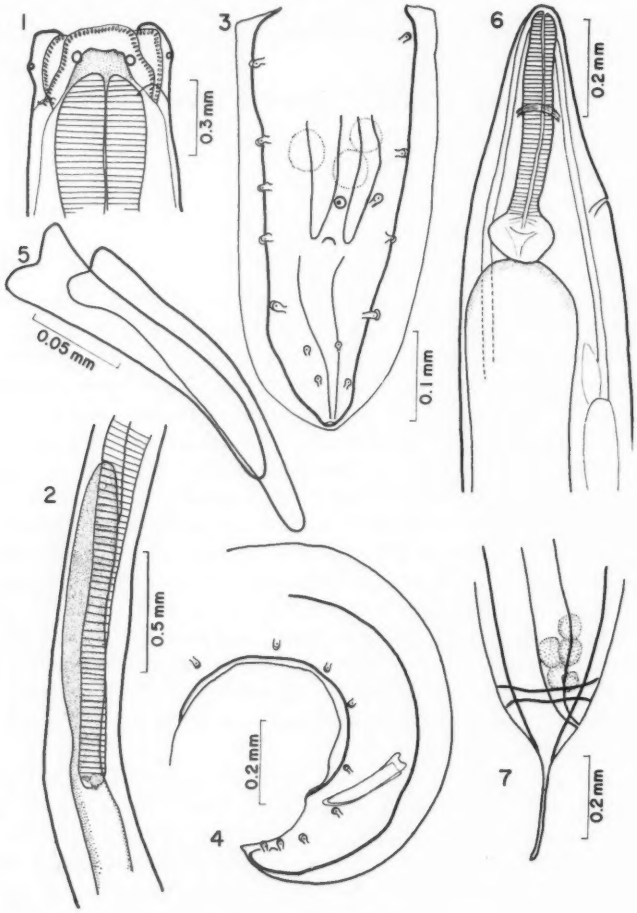
Male 16.89 to 21.2 mm long, 0.35 to 0.38 mm wide. Head diameter 0.1 to 0.14 mm. Oesophagus 2.67 to 2.9 mm long. Intestinal caecum 0.9 to 1.2 mm long. Nerve ring 0.52 to 0.57 mm, excretory pore 0.54 to 0.61 mm from anterior end. Tail (Figs. 3 and 4) short, 0.14 to 0.22 mm long. Caudal alae well developed. Six pairs preanal, one pair adanal, and three pairs postanal papillae. Median papilla on anterior lip of cloaca. Five pairs preanal papillae lie close together, the sixth pair far from them. Spicules 0.17 to 0.22 mm long (Fig. 5), slightly subequal, very short, cylindrical, broader at anterior end, narrow at posterior end. No gubernaculum.

Female 24.7 mm long, 0.49 mm wide. Head diameter 0.13 mm. Oesophagus 2.88 mm long. Intestinal caecum 1.3 mm long. Nerve ring 0.45 mm, excretory pore 0.48 mm from anterior extremity. Tail blunt, 0.19 mm long with a pointed button at tip. Vulva 8.43 mm, that is one-third, from anterior end. Eggs large and oval, 0.09 to 0.11 mm by 0.06 to 0.08 mm in size.

[1]Manuscript received March 25, 1960.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada. The parasites upon which this paper is based were collected by Dr. Robert E. Kuntz (CDR, MSC, USN, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan), a member of the U.S. Naval Medical Mission to East Pakistan in 1958. He was assisted by James Reese, HM, IC, USN, and Charles Knight, HCM, USN, also of NAMRU-2. Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, identified the amphibia.
[2]National Research Council of Canada, Postdoctorate Fellow.

Discussion

The present form belongs to the species *Amplicaecum cacopi* Chatterji, 1936 (2) but differs from it in the number and arrangement of anal papillae, in having six pairs of preanal papillae instead of five pairs, and in the arrangement of the postanal papillae. In the present form there are three postanal and one adanal while in *A. cacopi* there are four pairs of postanal papillae.



FIGS. 1–5. *Amplicaecum cacopi* Chatterji, 1936.
FIG. 1. Head, dorsal view.
FIG. 2. Junction of oesophagus and intestine showing intestinal caecum.
FIG. 3. Tail, male, ventral view.
FIG. 4. Tail, male, lateral view.
FIG. 5. Spicules.
FIGS. 6–7. *Thelandros* Wedl., 1862.
FIG. 6. Anterior region of female, lateral view.
FIG. 7. Tail, female, lateral view.

It differs also in possessing a median papilla which has not been observed in *A. cacopi.* Chatterji appears to have overlooked the median papilla and the sixth preanal papilla, which lies further away from the regular series of preanal papillae. These differences are considered variations within the species.

Oxyuridae Cobbold, 1865

Thelandros Weld. 1862

Thelandros sp. (Figs. 6–7)

Material: One female.

Host: *Rana cyanophylyctis.*

Location: Small intestine.

As this is only the second time this parasite has been recorded from anuran amphibia and as it constitutes a new record from East Pakistan, it seems desirable to discuss it.

Description

Worm small and thin, tapering towards each extremity, 5.4 mm long, 0.49 mm in maximum breadth. Cuticle thin, finely striated. Alae extend from anterior part of body and terminate a little behind the cloaca, leaving the caudal extremity free. Buccal cavity (Fig. 6) absent. Oesophagus 0.5 mm long, terminating in large spherical bulb, 0.1 by 0.13 mm in size. Intestine swollen at anterior end to form pear-shaped dilation. Three oesophageal intestinal valves projecting freely into lumen of intestine. Nerve ring situated 0.21 mm from anterior end. Excretory pore surrounded by ring of muscular tissue situated 0.41 mm from anterior end and anterior to oesophageal bulb. Vulva a conspicuous transverse slit slightly behind mid-body, 2.73 mm from tip of tail. Uterus coiled, extending anteriorly 0.68 mm from anterior end to region back of oesophageal–intestinal junction.

Tail (Fig. 7) about 0.3 mm long including blunt, rounded postanal portion of body and terminal spike which is 0.21 mm long. Uterus greatly distended with embryos and larvae.

Discussion

The present form differs from all the known species except *T. sexlabiaat* Ortlepp, 1933 and *T. numidicus* Seurat, 1918 in that the excretory pore lies anterior to the oesophageal bulb. It differs from *T. sexlabiata* (6) in having the vulva slightly posterior to mid-body instead of at the junction of the second and last body third. It differs from *T. numidicus* (7) in having a larger tail and in having the vulva slightly posterior instead of anterior to mid-body. It differs from *T. oswaldocruzi* Travassos, 1925 (9) from a Brazilian tree frog (*Hyla mesophaca*) in being smaller, in having the excretory pore anterior to the oesophageal bulb, and in having a smaller tail. As males are not available it cannot be assigned to a species.

Rhabdiasidae Railliet, 1915

Rhabdias ranae Walton, 1929

Material: Large number of females.
Host: *Rana tigrina.*
Habitat: Lungs.

Apart from an immature form of *Rhabdias escheri* Baer, 1930 (1) from a coecilian (*Uraeotyphlus oxyurus*) from Palni, South India, *Rhabdias* has not previously been recorded from the Indian Region.

Description

Parasitic form, 5.54 to 7.6 mm long, 0.3 to 0.38 mm wide. Mouth surrounded by six small lips. No papillae. Buccal cavity short with club-shaped swelling posteriorly. Oesophagus 0.34 to 0.37 mm long. Nerve ring 0.17 to 0.19 mm from anterior extremity. Intestine dark-colored, opening by way of cuticularized rectum to anus. Three rectal glands present. One pair cervical glands open just in front of posterior end of oesophagus and extend posteriorly along intestine.

Vulva opens to outside in posterior half of body. Vulva 2.6 to 3.2 mm from posterior extremity. Anterior loop of uterus from vulva 1.74 to 3.2 mm and posterior loop, 1.5 to 1.9 mm. Tail 0.21 to 0.39 mm long. Eggs elliptical and embryonated, 100 by 550 to 650 μ in size.

Discussion

The present form belongs to *Rhabdias ranae* (Walton, 1929) (10), but differs in being larger, in having a larger tail and larger eggs, and in the absence of two pairs of postanal lateral papillae.

Trichostrongylidae Leiper, 1918

Trichostrongylinae Leiper, 1908

Oswaldocruzia melanosticti sp. nov. (Figs. 8–14)

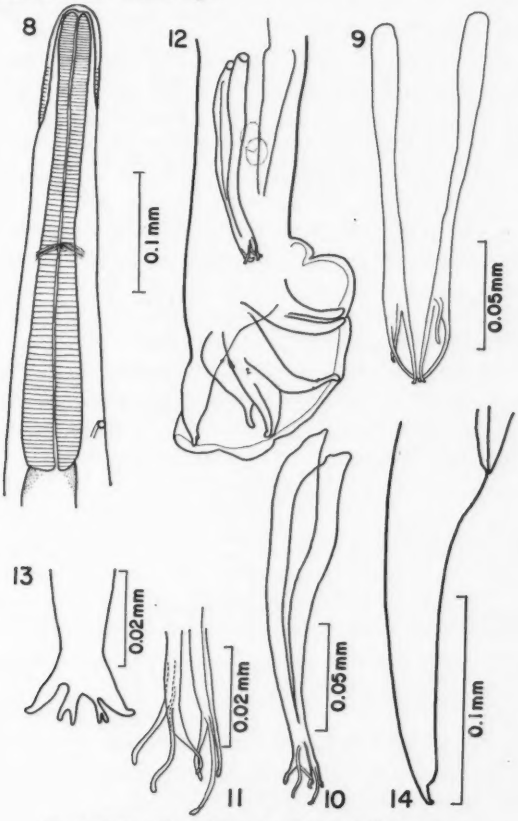
Material: Many males and females from *Bufo melanostictus* (type host).
Hosts: One male and two females from *Rana tigrina.*
Habitat: Small intestine.

Description

Body very slender, tapering towards both ends, anterior end curved ventrally. Transverse striations of cuticle absent except in region of cephalic cuticular inflation (Fig. 8), which is divided into two portions. Cervical papillae apparently absent. Body marked by numerous longitudinal cuticular ridges. Mouth opening triangular in frontal view possessing four papillae and two amphids. Lips absent. Oesophagus claviform. Both sexes taper gradually cephalad from cylindrical mid-body region; female tapers posteriorly more acutely than male; body ends in distinct spike.

Male 6.03 to 8.2 mm long, 0.13 to 0.14 mm wide. Anterior portion of cephalic cuticular inflation 0.03 to 0.04 mm long; posterior cephalic cuticular inflation 0.06 to 0.07 mm long. Oesophagus 0.39 to 0.46 mm long. Nerve ring 0.16 to

0.21 mm from anterior end of body. Excretory pore (Fig. 8) at 0.04 to 0.07 mm from posterior end of oesophagus. Spicules brown, similar, equal in size—0.17 to 0.19 mm long (Figs. 9–11)—the terminal portions of which divide into three terminal spike-like processes which are curved into hook-like processes. No prebursal papillae were observed. Ray formation of bursa copulatrix characteristic of genus (Fig. 12). Dorsal ray (Fig. 13) in its dorsal portion gives out two short side branches before bifurcating into two terminal branches which again bifurcate at their tips.



FIGS. 8–14. *Oswaldocruzia melanosticti* sp. nov.
FIG. 8. Anterior region of male, lateral view.
FIGS. 9–11. Spicules.
FIG. 12. Bursa, lateral view.
FIG. 13. Dorsal ray.
FIG. 14. Tail, female, lateral view.

Female 7.1 to 13.2 mm long, 0.21 to 0.22 mm wide. Anterior portion of cephalic cuticular inflation 0.03 to 0.04 mm long, thin posterior portion 0.06 to 0.08 mm long. Claviform oesophagus 0.44 to 0.49 mm long. Nerve ring at about posterior region of anterior third of oesophagus, lying 0.16 to 0.21 mm

from anterior extremity. Excretory pore 0.04 to 0.06 mm from posterior end of oesophagus. Tail (Fig. 14) 0.16 to 0.2 mm long, slender, conical, with spike. Vulva at 2.68 to 4.46 mm, nearly one-third from anterior extremity. Eggs elliptical, 550 to 750 μ by 500 to 600 μ in size.

Discussion

Lal (5) described *Oswaldocruzia* sp. from the intestine of *Bufo melanostictus* from India. One species, *O. hoepplii* Hsü, 1935 (3), has been described from frogs in Indo-China; one, *O. heparia* Koo, 1939 (4), from China; and one, *O. goezei* Skrjabin and Schulz, 1952 (8), from Europe and Asia.

The present form differs from *O. goezei* in the non-possession of narrow cervical alae extending approximately to the end of the oesophagus, in the peculiarities of the branch of the dorsal ray, and in the nature of the spicules, which in *O. goezei* are provided on the distal two-thirds with five chitinized processes joined by a membranous hyaline sheath.

It differs from *O. heparia* in the absence of a long, wide cephalic inflation, in the position of the excretory pore, which lies slightly anterior to the hind region of the oesophageal bulb instead of far from it, and in the peculiarities of the bursal rays.

It closely resembles *O. hoepplii* in its general characteristics but differs in the females being smaller, and in the characteristic nature of the posterior end of the spicules. In *O. hoepplii* the spicules are similar and equal and their terminal portions are divided into three processes each connected by a thin membrane. It differs from Koo's description of *O. heopplii* in the apparent absence of cervical papillae.

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TAXONOMIC POSITION OF MACROLAIMUS PAPILLATUS (RAHM, 1928) GOODEY, 1951, STATUS OF DIASTOLAIMUS RAHM, 1928, AND EMENDED DIAGNOSES OF CHAMBERSIELLIDAE AND CHAMBERSIELLA COBB, 1920 (NEMATODA)[1]

K. C. SANWAL

Abstract

Diastolaimus papillatus Rahm, 1928, is removed from synonymy with *Macrolaimus papillatus* (Rahm, 1928) Goodey, 1951, and transferred to the genus *Chambersiella* Cobb, 1920. The diagnoses of the family Chambersiellidae (Thorne, 1937) Sanwal, 1957, and the genus *Chambersiella* are emended to include forms with simple hairlike cephalic setae and postvulvar uterine branch. A key to species of the genus *Chambersiella* is given.

In attempting to arrive at a natural classification of animals, re-examination of the relationships of described species is quite as important as the discovery and descriptions of new species. In this paper, the taxonomic position of *Macrolaimus papillatus* (Rahm, 1928) Goodey, 1951 (4), is changed and the diagnoses of the family Chambersiellidae (Thorne, 1937) Sanwal, 1957 (8), and of the genus *Chambersiella* Cobb, 1920 (1), are emended.

The genus *Diastolaimus* was established by Rahm (5, 6) with *D. papillatus* as the type and only species. Rahm pointed out that this genus resembles the genus *Chambersiella* Cobb, 1920 (1), in various characters, for example, bristles at the cephalic extremity ("nao ciliadas" in his species) and the labial teeth. The species, however, differed from *Chambersiella rodens*, the only species in that genus at that time. Rahm referred to Cobb's remark that the genus *Chambersiella* included more than one species and that they differed in labial characters. Since Cobb did not describe these other species, Rahm, in view of the differences from *C. rodens*, reasonably placed his form in his new genus *Diastolaimus*. Later, Goodey (4) synonymized *Diastolaimus* with *Macrolaimus* Maupas, 1900. Ruehm (7) also lists *Diastolaimus* as a synonym of *Macrolaimus* after Goodey (4).

While studying a species of *Macrolaimus* associated with bark beetles, the author found a resemblance between the species *M. papillatus* and *Chambersiella bakeri* Sanwal, 1957 (8). Comparison of the other species of *Macrolaimus* with *M. papillatus* indicated that the species *papillatus* does not belong in the genus *Macrolaimus*. The similarities between *C. bakeri* and *M. papillatus* (= *Diastolaimus papillatus*), however, are so striking that synonymy of *Diastolaimus* with *Chambersiella* is indicated. The species *papillatus* is clearly eliminated from the genus *Macrolaimus* by the presence of hooklike anterior

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Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

rhabdions; the long, narrow, vaselike posterior region of the stoma; the posterior position of the amphid; and the shape of the male tail. Having these characters, *papillatus* should evidently be placed in the genus *Chambersiella*.

A fact that was overlooked by the author while describing *Chambersiella bakeri*, probably overlooked or not accepted by Thorne (9) while listing the species of *Chambersiella*, and overlooked by Goodey (4) while defining the genus *Chambersiella* and making the new combination *M. papillatus* is that Cobb (2) has synonymized the genus *Diastolaimus* Rahm, 1928 (5, 6), with the genus *Chambersiella* in his key to the genera of free-living nematodes. (This synonymy is also indicated in a footnote in Filipjev and Stekhoven (3).) Further, in the same key, Cobb places the genus *Chambersiella* under two groups: (*a*) those having one ovary and (*b*) those having two. Thus it will be seen that though only one species, *C. rodens*, was described, Cobb certainly intended the genus to include other species than *C. rodens* (with one ovary and "the strongest mandibles known in the genus"). These facts, although overlooked originally, lend support to the validity of *C. bakeri* and the emendment of the generic diagnosis of *Chambersiella* made by the author in 1957, and also the synonymy of the genus *Diastolaimus* with *Chambersiella*. Since Cobb (2) and Filipjev and Stekhoven (3) did not cite the name of the species, *Diastolaimus papillatus* Rahm, 1928, becomes *Chambersiella papillata* (Rahm, 1928) n. comb.

Recognition of the above synonymy necessitates slight modifications in the diagnoses of the family Chambersiellidae and the genus *Chambersiella*, as follows:

Family Chambersiellidae (Thorne, 1937) Sanwal, 1957, Emended

Diagnosis

Rhabditoidea. Lip region with six cephalic cirri or with hairlike setae; anteriormost rhabdions distinct from the rest; stoma broad at anterior end, gradually narrowing to a vaselike, long, and narrow channel posteriorly; posterior part of stoma surrounded by esophageal tissue; esophagus without a distinct median bulb but with a valvulated terminal bulb; one or two ovaries in the female, sometimes with a postvulvar uterine branch; amphids opening behind the broad anterior chamber of the stoma.

Type genus: Chambersiella Cobb, 1920.

Genus *Chambersiella* Cobb, 1920, Emended

Diagnosis

Cuticle finely striated, with lateral incisures; lip region with cephalic cirri or with hairlike setae; anteriormost rhabdions of stoma distinct from the rest and modified to form hooklike structures; posterior part of stoma forming a long, narrow, vaselike channel and surrounded by espohageal tissue; amphids opening behind anterior broad chamber of stoma; esophagus without median bulb but with a valvulated terminal bulb; ovaries one or two, sometimes with a postvulvar uterine branch; vulva slightly pre-equatorial to postequatorial;

testis single; male tail with several pairs of caudal papillae; spicules paired and not joined; gubernaculum present; tail of each sex with a dorsally hooked terminus.

Type species: *Chambersiella rodens* Cobb, 1920.

Other species: *C. bakeri* Sanwal, 1957.

C. papillatus (Rahm, 1928) n. comb.

Syn. *Diastolaimus papillatus* Rahm, 1928.

Macrolaimus papillatus (Rahm, 1928) Goodey, 1951.

C. rodens can be distinguished from *C. papillatus* by the possession of cephalic cirri and massive 'odontia' at the anterior extremity, by lack of a postvulvar uterine branch in the female, and by different size and proportions of the body. *C. bakeri* can be distinguished from *C. papillatus* by its smaller body length and possession of two ovaries and of cephalic cirri at the anterior extremity.

Key to Species of *Chambersiella*

1. With one ovary 2
   With two ovaries 3
2. Postvulvar uterine branch absent; cephalic cirri present ..... *rodens* Cobb, 1920
   Postvulvar uterine branch present; simple hairlike cephalic setae present ..... *papillatus* (Rahm, 1928) n. comb.
3. Cephalic cirri present ..... *bakeri* Sanwal, 1957

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NATURAL MORTALITY OF THE PINE SHOOT MOTH, RHYACIONIA BUOLIANA (SCHIFF.) (LEPIDOPTERA: OLETHREUTIDAE), IN ENGLAND[1]

P. HARRIS

Abstract

A population of *R. buoliana* infesting a young Scots pine plantation was followed through three generations. The most important causes of mortality in the population were an egg parasite, probably *Trichogramma evanescens*; the failure of the third instar larvae to establish themselves in the buds; two external parasites, *Scambus sagax* attacking the larvae in the autumn and *Exeristes ruficollis* attacking the larvae in the spring; three internal parasites, *Orgilus obscurator*, *Temelucha interruptor*, and *Eulimneria rufifemur*, attacking the larvae in the autumn but killing it in the spring after the trees were damaged; and a fungus, *Paecilomyces farinosus*, that usually killed the larvae in the autumn. The total proportion of the population killed by parasites and fungus was usually constant from one year to the next, though the abundance of *T. interruptor* increased.

Most of the annual fluctuation in abundance of the moth was caused by the differential survival of the third instar larvae. After a cool summer the third instar larvae were not developed until September and most of them failed to establish themselves in the buds and perished. On the other hand in a warm summer when the larvae developed earlier and attacked the buds in July, most of them were successful.

Introduction

The pine shoot moth (*Rhyacionia buoliana* (Schiff.)) is a more serious pest to pine reproduction in North America than in Western Europe. As it is a recent introduction to North America and is sparingly attacked by local parasites, it was hoped that the importation of its European parasites might reduce the amount of damage done. The establishment of several of these parasites has failed to achieve this purpose. This raises the question whether parasites are a primary cause of the relatively lower populations of the pine shoot moth in Europe. Harris (4) found that in Britain a large portion of the young larvae perished after a cool summer because their development was retarded in relation to the formation of resin canals so they were unable to establish themselves in the pine buds. The present paper is a report on egg, larval, and pupal mortality caused by parasites, resin, disease, and other factors in a Scots pine plantation in Southern England during a 3-year period.

Materials

Changes in the pine shoot moth population were recorded in a 7-year-old (planted 5 years previously with 2-year-old stock) plantation of Scots pine (*Pinus sylvestris* L.) on the sandy plateau of Bagshot Heath, Berkshire. Three

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very similar tortricids were found together in this stand, but *R. buoliana* was by far the most abundant. In a sample of 993 larvae collected in 1956, 91% were *R. buoliana*, 5.2% *Coccyx turionana* (Hbn.) (= *Rhyacionia turionana*), and 3.8% *Rhyacionia pinivorana* (Zell.); of 1278 larvae examined in 1957 over 97% were *R. buoliana*, under 0.3% *C. turionana*, and 2.7% *R. pinivorana*. The proportions were similar in 1958: in a sample of 106 larvae less than 5% were *C. turionana* and under 1% *R. pinivorana*. Fresh or well-preserved larvae of the three species can be separated by differences in the prolegs, but it is difficult to separate decayed larval remains, so the three species have been treated here as one population. The error introduced by this is not great because *R. buoliana* always comprised over 90% of the population.

The three species have similar habits, and differ mainly in their seasonal development. In Berkshire, *R. pinivorana* emerged in May–June, *C. turionana* in June, and *R. buoliana* in June–July. Both *C. turionana* and *R. pinivorana* overwintered in the penultimate instar, and *R. buoliana* in the third instar. *R. pinivorana* resumed feeding in the spring at the beginning of March, approximately a month earlier than the other two species. The three species are distributed widely over Europe, but they are abundant in different places. For example, *R. buoliana* is abundant in southeastern England and absent from northern Scotland. In contrast *C. turionana* is abundant in Scotland and sparse in the south of England (3). Less is known about the distribution of *R. pinivorana*; it seems to be widespread but not abundant in Southern England. The absence of *R. buoliana* from Scotland was attributed to its need for a warm summer to attack the buds before they are fully protected with resin (4).

Sampling Methods

Sampling the Egg and First Two Larval Instars

Unlike the later stages, the eggs and first two larval instars are not conspicuous in the field, so they were sampled by examining representative pine shoots before and after the needles were stripped off the shoots. The population was then expressed as the number of individuals per hundred shoots. These estimates were subject to a large sampling error as no more than 200 shoots could be examined in a day. In 1956 an attempt was made to increase the number of shoots that could be sampled and so reduce this error. It was noticed that few eggs were laid on the lower half of the tree, below the bracken level. Samples were taken only from shoots above the bracken, and the population estimate corrected for the proportion of shoots below the bracken on the assumption that there were no eggs or larvae on them. The shoots were sampled systematically at various heights and aspects of the tree from the terminal whorls of the lateral branches.

The reliability of this sampling method was checked at the end of September, when the larvae were easier to find. The number of larvae estimated on three groups of trees was compared with the actual population present. The estimated number of larvae per hundred shoots was 9.6, 9.7, and 14.0 while the actual number present was 8.85, 7.3, and 11.3 respectively. On the basis of

this comparison, all the population estimates made in August 1956 were reduced by 20%.

In 1957 and 1958 the shoots were collected by another method so that the samples would be more representative. The rows, the trees, the whorls, and the branches to be sampled were preselected from random number tables. Three complete branches were collected from different whorls on each tree (branches below as well as above the bracken were sampled) and 5 trees were sampled from a row of 20 trees. The deficiencies of small size of sample were partially overcome by frequent sampling, usually five or six times a week. The smooth transition between these estimates of the young larvae and the later field counts of older larvae indicated that the two methods gave comparative estimates (Fig. 1).

Sampling the Later Stages

The third instar larvae attack the buds and during the remainder of the year the infested shoots are marked by a conspicuous white deposit of resin. All the damaged buds on sample trees were collected for examination in the laboratory, where they were cut open and the living and dead larvae and pupae counted. The number of emerged adults was found by counting the empty pupal cases protruding from the infested buds or, when these dropped, the large, silk-lined buds containing a broken, mature larval head capsule. The total number of shoots on the sample trees was estimated from their stem diameter (5) and the population of pine shoot moth calculated as the average number of individuals on a shoot. The trees sampled were chosen at random from a row of 20 trees also randomly selected. The number of trees sampled was increased as the year progressed so that all samples would contain approximately a hundred living *R. buoliana*. To minimize any effect the removal of the shoot moth and its parasites might have, each new generation was sampled in a plot at least a hundred yards from the previous plot sampled. The plots, each containing 2000 trees, were as similar as possible and were checked to ensure that they had the same *R. buoliana* parent population.

Sampling Errors

The eggs and young larvae were difficult to find, so some were undoubtedly missed, while there was less chance of missing the older larvae as they were more obvious. This tended to obscure the mortality of eggs and young larvae or even result in an apparent population rise between the second and third instars. For example, in 1957 the estimates of third instar larvae were higher than the estimates of the earlier stages. The random sampling errors were less serious as they did not alter the population trend. The range in these egg and young larva estimates is shown graphically in Fig. 1.

Sampling error can be calculated for the later stages in the bud. Very little mortality occurred while the larvae were in hibernation in the buds between October, 1956, and April, 1957. This was checked by fitting a regression to the estimates of the healthy larvae. The slope of the line ($Y=2.878-0.00415X$) was not significant at the 5% level; thus these estimates can be used as replicates for calculating the sampling error. The coefficient of variation of the

standard deviation was 28% for a mean sample size of 89 larvae. The mean winter population after weighting by sample size was 2.58 ± 0.17 larvae per 100 shoots.

Seasonal Development of *R. buoliana*

The summer temperature has a strong influence on the seasonal development of *R. buoliana* and this, as shown in a previous paper (4), affects the survival of the third instar larvae. The mean monthly temperature at Silwood Park, Ascot (within five miles of the stand studied), for June, July, and August were respectively 56° F, 61° F, and 56° F in 1956; 60° F, 62° F, and 60° F in 1957; 57° F, 61° F, and 60° F in 1958. Thus the warmest summer was in 1957; it was slightly cooler in 1958 and coolest in 1956.

The effect of summer temperatures was that the adults emerged early in 1957, with emergence completed by the end of June, whereas in 1956 and 1958 it continued into the first half of July. Correspondingly, oviposition was completed earlier in 1957 and all eggs hatched by the beginning of August whereas in the other two years hatching continued until the end of August. In 1957 development continued to get further ahead of that of the other years as the summer advanced; the first larvae were found on 7 July, some 3 weeks earlier than in 1956, and over half the larvae were mining buds by 29 July, 5 or more weeks ahead of 1956. Development in 1958 was not as retarded as in 1956 and over half the larvae were in the buds by 18 August. The remainder of the life cycle was similar in the three years: the larvae went into hibernation in October and did not start feeding again until April.

Thus in 1957, the warmest summer, the adults emerged earlier, the eggs were laid earlier and hatched sooner, and the first two instars developed more quickly than in the other two years. Likewise development in 1958 was earlier than in 1956.

Survival of *R. buoliana*

General

Figure 1 shows the total number of eggs laid per hundred shoots and the number of apparently healthy larvae and pupae through the rest of the life cycle. Thus the graphs show the build-up of the population during oviposition and the subsequent survival throughout the rest of the generation. The decline shown in the graph is proportional to the mortality over any period, except that egg mortality is displaced until the end of oviposition.

In the following account all the population estimates are expressed as the number of individuals per hundred shoots unless otherwise stated. The first sample, of over 8300 shoots, taken on 1 June, 1956, had an average of 1.5 *R. buoliana* per hundred shoots. The emergence of parasites caused the population to drop to 0.8 by 4 July, and a later count showed that 24.5% of these were either parasitized or diseased so that 0.6 *R. buoliana* emerged as adults. They laid an average of 15.8 eggs per hundred shoots by 17 August. About 9% of these eggs were parasitized but oviposition in the second half of August more than compensated for this loss. On 28 August the population was estimated to

be 16.4, mainly of the second instar. These larvae attacked the buds in September, but most were unsuccessful in establishing themselves, and by 18 September only 5.4 remained. The reason for this large mortality was apparently the prolific production of resin by the buds in September (4). A few more larvae vanished in the second half of September, but no further loss occurred in October. An average of 4.7 larvae was found in the samples between 3 and 15 October.

Almost three-quarters of the third instar larvae perished before establishing themselves in a bud. A further 0.1 larvae were drowned in resin inside the buds and before the end of October others were killed by fungus and external parasites, leaving only 2.6 healthy larvae to overwinter. These larvae went into hibernation in October and suffered negligible mortality over winter. In early April, 1957, a few larvae vanished; perhaps the buds in which they were hibernating fell from the trees, as many of the buds damaged in the autumn were very loose and the growth of the other buds in April often dislodged them. Between 20 April and 16 June an average of 2.1 larvae remained alive. Most of the difference between this figure and the 1.5 adults that emerged by 1 July (from a sample of almost 6900 shoots with an average of 0.77 pupal cases and 0.72 empty silk-lined buds) was caused by parasitism though a small number were killed by a fungus disease.

At the end of the oviposition period (5–12 July, 1957), there was an average of 15.4 eggs and larvae per hundred shoots. There was no egg parasitism and, in contrast to the previous year, almost all the larvae established themselves in the buds because they attacked before the buds developed an adequate

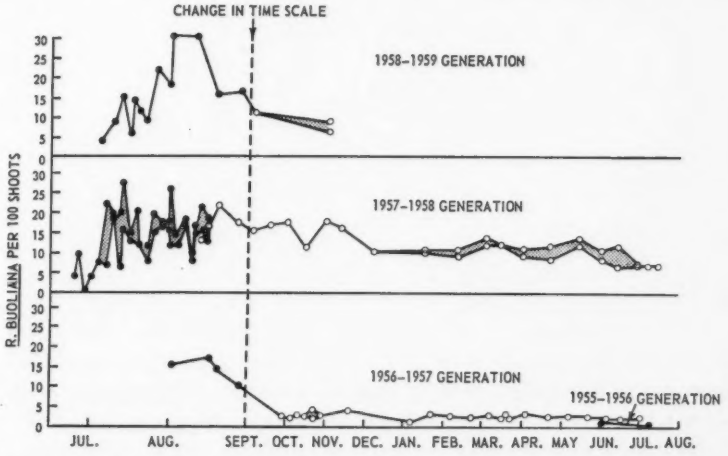

FIG. 1. Number of *R. buoliana* per hundred pine shoots in successive estimates between June 1956 and November 1958. The area between two estimates made on the same day is stippled to provide a visual estimate of sampling error. The early stages of the moth, from late June to late August, were estimated from random samples of shoots and are shown by a black dot. The later stages which were estimated from all the shoots on randomly selected trees are shown by a white dot.

resin defense system. In fact the population estimates increased slightly once the larvae were in the buds because, being conspicuous, more were discovered. Thus, between 14 August and 11 November there was an average of 18.1 larvae in the buds. These were attacked by fungus and external parasites, leaving an estimated 12.8 healthy larvae to overwinter. Only 10.0 of these could be found in the samples taken during December, January, and February, but from 3 March to 12 May the average population found was 11.5 per hundred shoots. Thus there was apparently a loss of 1.3 larvae at the end of November. Little further reduction occurred in the population until the internal larval parasites emerged at the end of May and in June. An average of 6.35 moths per hundred shoots was estimated to have emerged from over 3500 shoots on 11 July (3.58 pupal cases, 1.67 empty silk-lined buds, and 1.10 healthy pupae).

The larger emergence of moths in 1958 resulted in more eggs on the shoots than in the other two years. Between 31 July and 10 August the population index of eggs was 30.4. About 17% of these eggs were parasitized and did not hatch. By 10 August the index of 24.9 was composed, except for a few eggs, of larvae feeding on the needles. Larval development was intermediate between that of the previous two years, so that they were less successful in establishing themselves in the buds than in 1957, but more successful than in 1956. The estimated number of larvae in the buds on 1 September, 1958, was 11.8. Hence just under half the larvae had perished by failing to establish themselves in the buds. The number surviving fungus and parasite attacks to overwinter was 8.7 per hundred shoots.

To conclude, most of the variation in the percentage of *R. buoliana* surviving to maturity from one year to the next was caused by the survival of the third instar larvae. For example, the number of eggs laid per shoot in 1956 and 1957 were very similar, but in 1957 almost four times as many larvae established themselves in the buds as in 1956. This differential remained for the rest of the year in spite of heavy mortality from fungus and parasites, with the result that just over four times as many adults emerged in 1958 as in 1957. The survival of the third instar larvae seems related only to the time of year they attack the buds initially and not to the density of the population. The effect of this is to inhibit the development of a major outbreak on Scots pine in a cool summer. On other species of pine a greater or smaller proportion of the population is killed, depending on how easily the resin is removed by the larva. For example, Corsican pine is less severely damaged than Scots pine, and they both suffer more damage after a warm summer than a cool one (4). Also, stands in the Brecklands of East Anglia and Bagshot Heath in Berkshire, where the summers are hotter than elsewhere in Britain, are more susceptible to outbreaks. Thus the higher losses in North America may be partly related to the warmer summers and to the species of pine grown.

Brookes and Brown's (1) observation that pine shoot moth infestations collapse as stands mature and branches interlace may also be explained by the resin defense of the buds: as the stand matures, the resting buds develop

earlier (P.F. Wareing 1957, in litt.) so that they presumably develop an adequate resin defense earlier. The development of the larvae, on the other hand, is probably retarded as the temperature in the buds and needle sheaths will be lower as the branches shade one another.

Adult Fecundity

Fecundity was found by dividing the number of eggs laid on the pine shoots by half the number of moths emerging: the sex ratio of the moths is approximately unity. The number of females emerging and the number of eggs laid on a hundred shoots are shown below. The estimates of eggs laid per moth were increased 60% to correct for the annual increase in the shoot number of the stand (4).

Year	No. females per 100 shoots	No. eggs per 100 shoots	Eggs per female
1956	0.315	26.24	83
1957	0.745	29.44	40
1958	3.175	48.64	16

The estimates of the number of eggs per female may be a little low as some eggs were probably not found, but the 1956 average is close to the 75 eggs per female given by Brookes and Brown (1). In the other years the total number of eggs laid increased only a little with the larger number of females, so that the average number per female decreased. This decrease may be due entirely to differences in adult mortality and summer weather, but further evidence that there may be a restriction of the number of eggs that the moths will lay in a pine stand is provided by Brookes and Brown (1), who found similar numbers of eggs per square inch of shoot on trees of different sizes in adjacent stands. Likewise the stand studied had an adult population of 0.6 per 100 shoots which laid an average of 15.6 eggs per hundred shoots by 2 August, 1957. An adjacent older Scots pine stand with a smaller parent population had 16.9 eggs per hundred shoots when sampled on 15 August; a lodgepole pine plantation with approximately 1.7 adults had 15.4 eggs per hundred shoots when sampled on 5 August. It is speculated that contacts with places where other moths have rested may stimulate dispersion. This may also explain why small trees in a stand have the same number of eggs per hundred shoots as large trees (5). However, in North America there seems to be no restriction over the density of eggs laid.

Egg Fertility

In the laboratory *R. buoliana* lays eggs within a few days of emergence whether the eggs are fertilized or not. The infertile eggs remain yellow and subsequently shrivel whereas the fertile eggs turn brown. Few shrivelled eggs were found in the field, suggesting that most moths mate successfully. In 1956, 0.3% of the 340 eggs examined over the oviposition and incubation periods were shrivelled; in 1956, 5.2% of 210 eggs were shrivelled, while in 1958 there were no shrivelled eggs in the 330 sampled. The high mating success

of the moths was verified by catching 50 females in the stand at least a week after emergence and caging them individually. Twenty-one died without laying, 27 laid fertile eggs, and 2 laid a single infertile egg each.

Egg Mortality

Parasitism by a trichogrammatid, probably *Trichogramma evanescens* Westw., caused most of the egg mortality. The parasitized eggs were black and could be readily distinguished from the yellow or pale reddish-brown color of healthy eggs. Percentage parasitism varied considerably each year in the stand: in 1956, 9.1% of the eggs examined were parasitized, in 1957 no parasitized eggs were found in this stand, while in 1958, 16.7% of the eggs were parasitized. This variation probably depends on the succession of alternate hosts earlier in the year.

Larval and Pupal Mortality

The larval mortality of the first two instars could only be determined by the number of dead larvae found on the needles. It could not be checked by the difference between the number of eggs and third instar larvae, as the estimates of the early stages were known to be low. Dead larvae may drop off the needles and be missed; however, it seems unlikely that a large mortality on Scots pine in the three years would have been unnoticed. In 1956, only 1 of 229 first and second instar larvae examined was mummified; 228, the remainder, were apparently healthy. In 1957, of the 581 larvae examined, 565 were healthy, 11 mummified, 4 dead in resin, and 1 partially eaten by a chrysopid.

A large mortality occurred in some years while the third instar larvae were establishing themselves in the buds. In 1956 the population index fell from 16.4 to 4.7 larvae, and in 1958 from 24.9 to 11.8 larvae. In 1957, however, the index rose from 15.4 to 18.2. This apparent increase was probably the result of greater efficiency in finding the larger larvae; it was present in all years, but masked by the large mortality in 1956 and 1958. The drop from needle-feeding to bud-feeding larvae was therefore 19.6 to 4.7 in 1956, and 29.8 to 11.8 in 1958 but this correction has not been introduced into Table III for these years.

The few larvae embedded in resin were found only during the initial attack on the buds. The average number of larvae embedded in 1956 was 0.09 per hundred shoots. A total of 4.7 larvae were in the buds during the first half of October, so that 4.6 were successfully established.In 1957 the average number was 0.06 per hundred shoots and the average number successfully established was 18.1 between 2 August and 11 November. In 1958 no larvae were found in the resin but a smaller number of larvae was sampled.

A fungus, *Paecilomyces farinosus* (Dicks ex Fr.) Brown and Smith, was isolated from mummified larvae collected in the field. Brown and Smith (2) report that *P. farinosus* attacks many kinds of insects. It is most active around 24° C and practically inactive below 8° C. The activity of the fungus on *R. buoliana* was tested by inoculating larvae with spores from a culture and keeping them at various temperatures. None of the infected larvae survived.

At 30° C they all died in 3–4 days, at 20° C in 5–6 days, and at 5° C some of them survived for over a month, but died within 3 days when brought to room temperature.

In the field the diseased larvae became wizened and mummified and their remains slowly disintegrated within the bud until they were no longer distinguishable from the resinous frass and fragments of bud scale. The disintegration was helped by a scavenger mite that fed on the dead larvae. The mites did not attack the living larvae but it is possible that they might have spread the fungus as they were often found wandering over the buds and shoots.

An epidemic of fungus occurred at the end of September, 1956. The number of dead larvae found between 3 and 23 October was 1.25 per hundred shoots. The number of larvae that entered the buds successfully was 4.61, so 3.36 survived the disease. Those killed by the fungus in September gradually disappeared during the winter, and by March scarcely any evidence of them remained (Fig. 2).

In 1957 the larvae entered the buds much earlier and the fungus appeared at the beginning of September. The number of dead larvae found between 2 September and 11 November was 1.1. Thus of the 18.1 larvae in the buds,

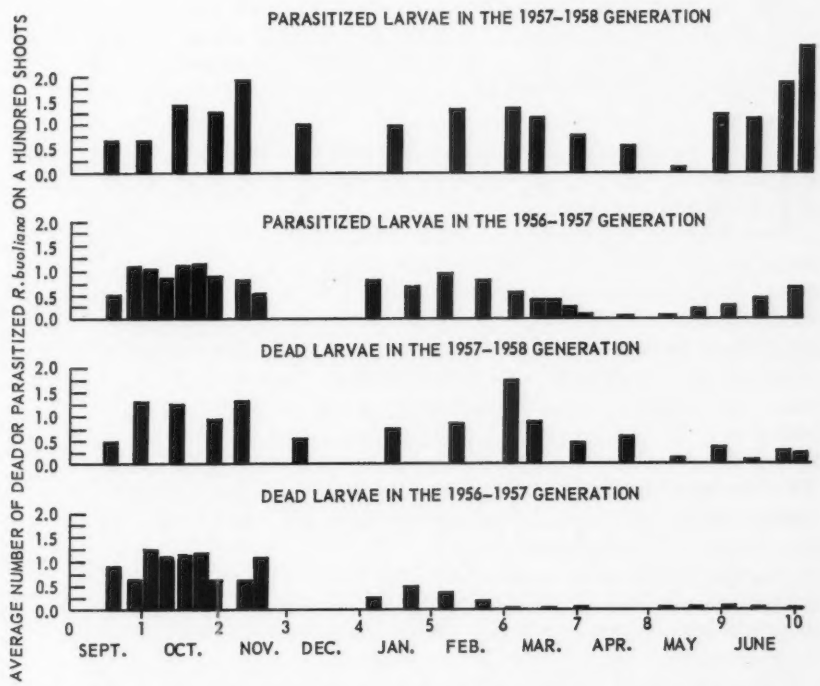

FIG. 2. Average number of dead and parasitized *R. buoliana* larvae per hundred pine shoots in successive samples of the 1956–1957 and 1957–1958 generations.

17.1 larvae should have survived. Unlike 1956, however, a drop in the number of healthy larvae was found in November and December and the number of mummified larvae was maintained over winter (Fig. 2). This suggests that a second outbreak of the fungus occurred at the end of November. The total number killed by the fungus was possibly 5.3, as only 12.84 larvae per hundred shoots survived. In 1958 an average of 1.5 mummified larvae were found in a sample of over 2200 shoots collected on 31 October. No other samples were taken to determine the full extent of the outbreak, but for purposes of calculation it is assumed that 10.3 larvae survived.

Several species of hymenopterous parasites attacked in the autumn but only *Scambus sagax* (Htg.) and *Habrocytus eucerus* (Ratz.) killed the larvae before winter; the latter, probably a secondary parasite, occurred in the ratio of 100 *Scambus* to 9 *Habrocytus*. These parasites attacked at the same time as the fungus.

In 1956 an average of 1.1 parasites per 100 shoots was found between 27 September and 23 October; by subtraction from the number surviving the fungus, this leaves an average of 2.3 larvae to overwinter. However, the average of all the winter samples was 2.58 ± 0.17 larvae and only 0.74 parasites were found between 29 October and 22 January. The emergence of these parasites started in March and was completed by April (Fig. 2). In 1957 the peak attack of parasites was again in September, but a few *S. sagax*, about 0.2, attacked the larvae in August and emerged in September. In the peak attack an average of 1.2 parasites was found between 2 September and 11 November and the combined attacks left 11.5 larvae to overwinter. In 1958 the number of parasites found in a single sample taken at the end of October was 1.3, which leaves 8.7 larvae to overwinter. However, the actual number of larvae found was only 7.7.

On emergence in the spring, *S. sagax* attacked the larvae a second time, so that this parasite could complete three generations a year on *R. buoliana* alone. However, females were not produced from the autumn and winter generations on *R. buoliana*, so the effectiveness of this parasite was limited. This was shown by collections of larvae reared in the laboratory, which produced 122 males and only 4 very small females. I suspect that these females were from *C. turionana* and *R. pinivorana*, which overwinter as slightly larger larvae and which form about 5% of the population in the stand. Thus, the spring generation of *S. sagax*, which has a more normal sex ratio, is small. The number of larvae killed by *S. sagax* decreased with the abundance of *R. buoliana*. For example, in 1956, with 5.0 larvae per hundred shoots available for attack, 21.9% were parasitized; in 1958, with 11.8 larvae available, 11.0% were parasitized; and in 1957, with 18.1 larvae available, only 7.4% were parasitized. Perhaps the abundance of the parasite is limited by that of the alternate host, which means it is likely to be more influential at low shoot moth densities than high ones.

Many species of parasite attack the young needle-feeding larvae. They overwinter in the first instar inside the host and emerge to kill it just before pupation in the early summer. The percentage parasitism by these parasites

was found by dissecting the hibernating *Rhyacionia* larvae. The parasites were not identified to species, but divided into four groups that separated the common species: the mandibulate parasite larvae were characteristic of *Orgilus obscurator* (Nees) (6); the caudate larvae with dark egg capsules, not absorbed, characteristic of *Eulimneria rufifemur* (Thoms.); the caudate larvae with white egg capsules, often absorbed (Juillet, personal communication), characteristic of *Temelucha interruptor* (Grav.); and the dipterous larvae, probably *Actia nudibasis* Stein. Some multiple parasitism occurred, particularly in 1958 when 5% of the host larvae were attacked by both *Temelucha* and *Orgilus*, 5% by *Eulimneria* and *Temelucha*, and 3% by *Eulimneria* and *Orgilus*. In these situations *Temelucha* survives over *Orgilus* (Juillet, in preparation), and *Eulimneria* was assumed to survive over the other species (see Table I).

TABLE I

Year	Number *Rhyacionia* dissected	Percentage parasitism				
		Mainly *Orgilus obscurator*	Mainly *Temelucha interruptor*	Mainly *Eulimneria rufifemur*	*Actia nudibasis*	Total
1955–56	78	5.1	12.8*		2.6	20.5
1956–57	151	14.6	8.6	6.0	1.3	30.5
1957–58	200	7.5	16.5	6.0	0	30.0
1958–59	100	14.0	26.0	18.0	0	58.0

*Egg color was not recorded for five of the parasites found; the other five were *Eulimneria rufifemur*.

Total parasitism increased during the three years. Most of this increase was caused by *Temelucha*. The abundance of both *Orgilus* and *Eulimneria* fluctuated annually and *Actia nudibasis* was not common. It is not known whether *Temelucha* would continue to increase until it had brought about the collapse of the host population.

Most of the spring and summer mortality was due to parasitism and was found by counting the parasite larvae and cocoons in the infested buds. The figure obtained is a combination of the internal parasites that overwintered in the host and of other parasites that attacked in May and June, such as *Exeristes ruficollis* (Grav.) and *Pimpla turionellae* (L.). *E. ruficollis*, an external parasite of the late instar larva, provided some difficulty as it usually emerged before the host and did not leave noticeable signs of its presence in the bud. Thus its emergence was marked by a seemingly inexplicable loss in the *R. buoliana* population. For this reason the method is far from ideal for finding the number and species of parasites. The obvious alternative of rearing collections in the laboratory proved even less satisfactory as the collections either became heavily contaminated with the fungus *P. farinosus*, or died from dehydration.

There were 1.54 shoot moths per hundred shoots in the first sample of the 1955–56 generation taken on 1 June. In the next sample, taken on 4 July, 16% of the population was killed by parasites, 8% mummified (killed by fungus), and 24% had disappeared, probably due to the emergence of *E. ruficollis*. The winter dissections showed that 21% of the larvae were

parasitized, but as *E. ruficollis* does not distinguish hosts that contain internal parasites from those that do not (Juillet, 1959, in preparation), 24% of these would fail to emerge. Thus there is no discrepancy between the 16% killed by these parasites in the summer and the 21% parasitism in the winter. Later samples showed that 21% of the pupae were parasitized by *P. turionellae* and 3.5% were diseased. Thus a total of 0.8 *R. buoliana*, or 51%, were killed by parasites during the spring and summer and only 8% by fungus.

In the 1956–57 generation 2.6 larvae overwintered, but only 2.1 were found feeding after the middle of April. The loss of 0.5 larvae may be due to some of the buds containing hibernating larvae falling off the trees in early spring. After the middle of April the total number of *R. buoliana*, dead and alive, found in the samples remained constant until after adult emergence at the end of June. For example, by 1 July 1.5 moths emerged, 0.6 larvae and pupae were killed by parasites, and 0.04 mummified. The figure for parasitism includes the external parasites that did not emerge until after the moth, at the beginning of July in 1957. They were, however, not as abundant as in the previous year, and in rearings they only accounted for 1% of the *Rhyacionia* population, equally divided between *E. ruficollis* and *S. sagax*. The pupal parasite, *P. turionellae*, also killed about 1% of the population. Thus a total of about 29% of the population was killed by parasites between the middle of April and the end of June and no other causes of mortality were important.

In the 1957–58 generation most of the 11.5 larvae that overwintered could be traced until the middle of June. After the middle of June some *R. buoliana* disappeared, again probably due to the emergence of *E. ruficollis*. Thus on 11 July only 9.3 individuals could be traced: 6.35 moths emerged, 2.54 killed by parasites (0.51 by pupal parasites), and 0.39 mummified (mostly pupae). Thus parasitism accounted for a possible 41% and the fungus only 3% of the spring and summer mortality.

The mortality caused by internal larval parasites, external parasites, and pupal parasites on the basis of these calculations are compared in Table II. It is debatable whether all missing individuals should be attributed to *E. ruficollis* or to some other cause, such as predation.

TABLE II

	% parasitism				
	Internal				
Year	By dissection	By cocoon count	External	Pupal	Total
1955–56	21	16	24	11	51
1956–57	31	27	1	1	29
1957–58	30	18	19	4	41

Mortality Table

The survival and mortality of the *R. buoliana* population are summarized in Table III. It was assumed that the estimates of adult emergence and the number of dead eggs, larvae, and pupae found in the samples were correct.

TABLE III

The number of *R. buoliana* eggs, larvae, and pupae on a hundred shoots and the approximate mortality at various stages in the life cycle

Stage	Mortality factor	1955–56		1956–57		1957–58		1958–59	
		No. per 100 shoots	% mortality	No. per 100 shoots	% mortality	No. per 100 shoots	% mortality	No. per 100 shoots	% mortality
Egg				18.1		19.2		29.9	
	Not hatching				9.4		5.2		16.7
Larvae in needles				16.4		18.2		24.9	
	Failure to enter buds				68.3		0.6		47.4
Larvae in bud				5.0		18.1		11.8	
	Drowning				1.8		0.3		0
	Fungus				24.9		29.3		12.7
	Parasites				21.9		7.4		11.0
Larvae overwintering		1.5		2.6		11.5		8.7	
	Falling from trees		—		16.7		0		—
	Parasites		51.3		24.0		41.4		>58.0
	Fungus		7.8		1.5		3.4		—
Adults emerging		0.63		1.49		6.35		<3.6	

The other estimates, such as the number of eggs laid, were adjusted to remove sampling discrepancies. Hence the table only indicates the trend of mortality and does not give the precise number that died at any stage.

The table shows that the annual variation in the number of eggs laid on a hundred shoots is much less than the variation in the number of adults emerging: the density of the eggs varied by less than a factor of 2 whereas the density of the emerging adults varied by a factor of 10. Most of this variation in adult emergence was determined by the success of the third instar larvae in establishing themselves in a bud. Parasites and the fungus often killed larger proportions of the population but they had less influence on the annual fluctuations of the population as they killed a similar proportion each year. The survival of the third instar larvae, on the other hand, varied widely from year to year. There was a negligible mortality in the warm summer of 1957; 47% failed to enter the buds in the cooler summer of 1958, and 68% failed to enter the buds in the still cooler summer of 1956.

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THE CAPSULE AND ITS RELATION TO THE EMBRYOGENESIS OF THE ICHNEUMONID PARASITOID MESOLEIUS TENTHREDINIS MORL. IN THE LARCH SAWFLY, PRISTIPHORA ERICHSONII (HTG.) (HYMENOPTERA:TENTHREDINIDAE)[1]

JOAN F. BRONSKILL

Abstract

Eggs of the parasitoid *Mesoleius tenthredinis* Morl. from British Columbia develop and hatch in approximately 120 hours when reared at 23±0.5° C and a relative humidity of 72±2% within field-collected larch sawfly (*Pristiphora erichsonii* (Htg.)) larvae from British Columbia and Newfoundland, and within laboratory-reared *P. erichsonii* larvae from British Columbia. However, they develop normally for only 70 to 80 hours within field-collected larvae from Manitoba as encapsulation of the developing eggs occurs and development ceases in the late germ band stage. The capsule is formed by an accumulation of host blood cells. During the formation of the capsule the innermost blood cells become flattened, fibrous, and enucleated, and eventually form a mass of concentric sheaths of non-cellular material. Thus, the definitive capsule is both cellular (outer region) and non-cellular (inner region). Histochemical tests indicate the presence of mucopolysaccharide in the capsule. It is suggested that the capsule inhibits the embryonic development of the parasitoid by interfering with its oxygen supply.

Introduction

By 1945 it became apparent that the introduced ichneumonid parasitoid *Mesoleius tenthredinis* Morl. was no longer effective in controlling an outbreak of the larch sawfly, *Pristiphora erichsonii* (Htg.), in Ontario, Manitoba, Saskatchewan, Alberta, and in some sections of the Lake States (14). A high proportion of the eggs were encapsulated in the host larvae and consequently failed to hatch (13). Muldrew (14) reported that the encapsulation reaction had reduced effective parasitism in central Canada from about 75% in an earlier outbreak to about 5% in the latest. Maw (10) also observed this immune reaction in 29% of the larch sawfly larvae from Britain. Though the resistance of the sawfly is still in doubt in Quebec (14), *P. erichsonii* has remained susceptible to the parasitoid in British Columbia (11), the Maritimes (19), Newfoundland (15), and New York State (2).

Many entomophagous insects can develop normally only in certain hosts. It is well known that a biological immune reaction is responsible for the inhibition of their development within unsuitable hosts. This immune reaction may manifest itself in the form of an agglomeration of host cells about the eggs of the parasitoid (1, 7, 8, 12, 18, 21, 22, 23, 24, 25). Data (13, 14) suggest that *P. erichsonii* has only recently acquired this type of immune reaction.

Muldrew (13) observed that the embryonic development of *M. tenthredinis* eggs began shortly after they were laid in the host larvae and continued for a period of 3 to 4 days at which time embryonic development in encapsulated

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Contribution from the Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

eggs was abruptly inhibited. He also noted that encapsulated, partially developed eggs remained viable for 7 months within the capsule, and only completed development and hatched in Ringer's solution after their dissection from the host larvae.

In this study the embryonic development of *M. tenthredinis* in larvae of *P. erichsonii* from Newfoundland, Manitoba, and British Columbia is compared, and the histological nature of the capsule and its relation to embryogenesis of this parasitoid is discussed.

Materials and Methods

Field-collected, first to third instar larch sawfly larvae from Newfoundland, Manitoba, and British Columbia were used in this study, as well as laboratory-reared sawfly larvae that originated in British Columbia. Fourth and fifth instar host larvae were exposed to mated, gravid females of *M. tenthredinis* obtained from sawfly cocoons collected in British Columbia. The parasitized larvae were incubated individually in glass vials (3 in. long and 3/4 in. inside diameter) at $23 \pm 0.5°$ C and a relative humidity of $72 \pm 2\%$. Each vial was supplied daily with a fresh twig of *Larix laricina* (Du Roi) K. Koch. Plugs of copper screening (20 mesh/in.) provided the necessary ventilation for the parasitized larvae in the vials.

Host larvae from British Columbia and Manitoba were dissected in Ringer's (9, p. 77) solution at approximately 12-hour intervals throughout the first 144 hours after parasitization. Dissections of larvae from Manitoba were continued at 4-day intervals from 144 hours to 52 days following exposure to the parasitoid. As the sawfly larvae from Newfoundland were being tested only for the presence or absence of the immune reaction, they were dissected daily from 3 to 8 days after exposure to *M. tenthredinis*.

Carnoy's (4, p. 44, No. 89), alcoholic Bouin's (4, p. 325, No. 754), and Helly's (9, p. 33) fixatives were used to preserve the 396 embryos obtained from these dissections. After 5 minutes of fixation, each normal embryo was pricked once and the chorion removed, while each encapsulated embyro was pricked three times and the capsule and chorion left intact. Then the normal and encapsulated embryos were placed in fresh fixative for 5 ± 1 hours. Eighty per cent ethanol was used to wash the fixative from the Carnoy's and alcoholic Bouin's preserved embryos, and 50% ethanol in washing the Helly-fixed material. Fixed embryos were prepared for sectioning by dehydrating in ethanol and clearing in benzol before impregnation with Tissuemat[2] (melting point 54–56° C). All embryos were sectioned at 4 μ.

Delafield's haematoxylin and eosin were used to stain sections of normal and encapsulated embryos for the determination of anatomical and histological relationships. Sections of encapsulated embryos were stained by the periodic acid–leuofuchsin (6) routine (with control sections treated in 1% aqueous malt diastase[3] for 1/2 hour, at 37° C for glycogen removal) for the presence of periodic

[2]Fisher Scientific Company, Toronto 8, Ontario.
[3]Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

acid – Schiff (PAS) reactive substances, and with toluidine blue (17, p. 432) in McIlvaine's (9, p. 263) buffer at pH 2.6 and 5.0 (with control sections treated in ribonuclease[3] (17, p. 473) and bovine hyaluronidase[3] (1 mg/ml) in phosphate buffer at pH 7.0 for 3 and 24 hours) for the detection of metachromatic components. The detection of polysaccharides and carbohydrate–protein complexes was possible with these histochemical procedures. The scarcity of encapsulated embryos prevented further histochemical examination of the capsule.

A Leitz Ortholux microscope with an Aristophot photomicrographic attachment was used to prepare the figures of the living and sectioned embryos. Figures 5 to 8 were made from embryos fixed in Carnoy's fluid and stained in Delafield's haematoxylin and eosin.

Results and Discussion

In the larvae of *P. erichsonii* from British Columbia and Newfoundland, the eggs of *M. tenthredinis* develop and hatch in approximately 120 hours when incubated at $23 \pm 0.5°$ C and a relative humidity of $72 \pm 2\%$.

Twenty-eight of the 65 host larvae from Newfoundland that were exposed to gravid females of *M. tenthredinis* were parasitized. The parasitoid was in the embryonic stage in 12 of these because they were dissected before the normal hatching time of *M. tenthredinis* (5 days). The other 16 parasitized host larvae were dissected from 5 to 8 days after exposure and contained active first instar larvae of *M. tenthredinis*. As there was no sign of inhibition to parasite development in the host larvae, it would seem that *P. erichsonii* in the region of Lake St. John, Newfoundland, the district in which these larvae had been collected, are still susceptible to *M. tenthredinis*.

A comparison of the embryonic development of *M. tenthredinis* in the larch sawfly from British Columbia and Manitoba showed that development in the host larvae from both regions was similar for 70 to 80 hours. At this time encapsulation occurred within the host larvae from Manitoba. Of the 48 encapsulated embryos dissected from Manitoba larvae between 76 hours and 52 days after parasitization, 40 (83%) were at the 70- to 80-hour stage of development, 4 (8%) were inhibited at an earlier stage, while 4 (8%) exceeded the 70- to 80-hour stage of development. These data indicate that encapsulation is the prime inhibitor of embryonic development and usually occurs at a definite time after the egg is laid.

Before encapsulation and the inhibition of embryonic development, the embryo develops normally to the late germ band stage (Fig. 4, *gb*). At this stage the segmented germ band has reached its greatest length, covering the entire ventral and lateral surfaces of the egg and extending over the posterior pole with its proctodaeum lying slightly dorsad (Fig. 4, *pr*). In the cephaloventral region of the germ band, the stomodaeum (Fig. 6, *st*) and gnathal region (*gn rg*) are clearly visible. The mesoderm (Fig. 6, *mes*) has differentiated; however, the mid-gut rudiments, as yet, have not enclosed the remaining yolk, nor have the ectodermal and mesodermal tissues extended dorsad over

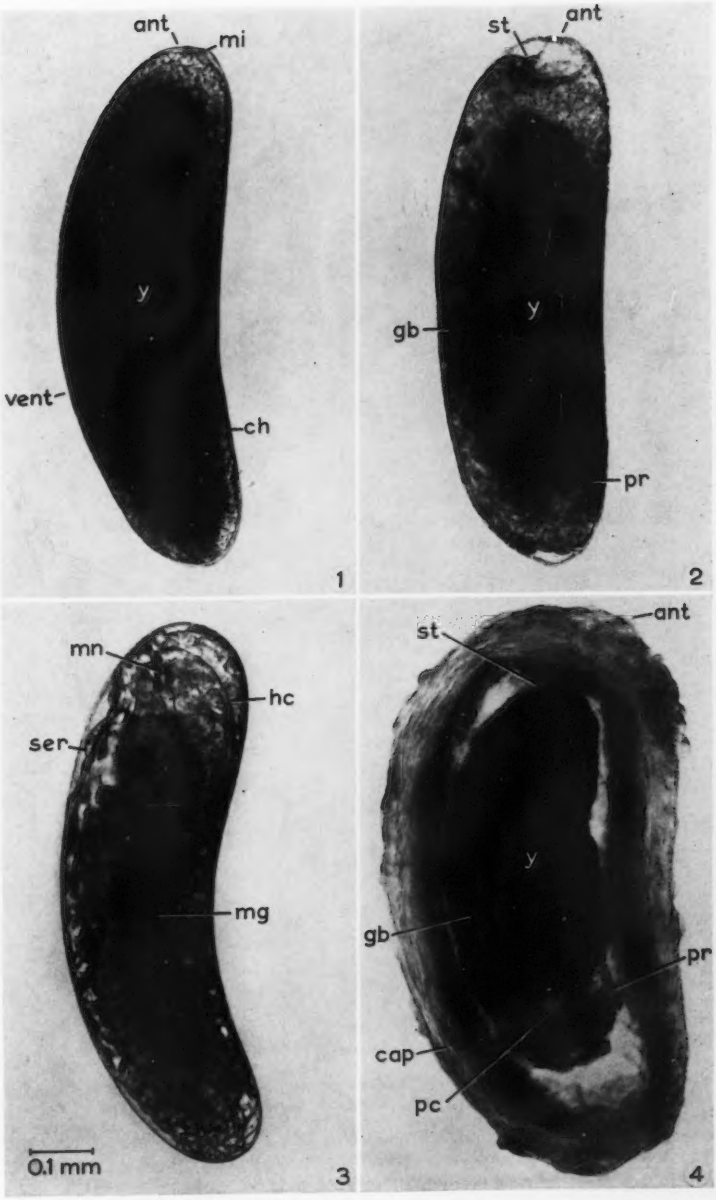
it. The pole cells (Fig. 4, *pc*) lie just ventrally to the anterior end of the proctodaeum. The embryo and remaining yolk are enveloped by the serosal membrane (Fig. 6, *ser*). Much histogenesis and organogenesis would have to occur before the encapsulated embryo completed its embryonic development.

Figures 1 to 4 illustrate the effect of the capsule on the embryonic development of *M. tenthredinis* in *P. erichsonii*. Figure 1 shows an egg of *M. tenthredinis* dissected from a British Columbia host larva a few minutes after oviposition. The translucent white egg is 0.8 mm long and 0.3 mm wide at its widest point (5). The embryo in Fig. 2 was dissected from a British Columbia host larva 75 hours after deposition. The embryo is in the late germ band stage; its segmented germ band, stomodaeum, proctodaeum, and yolk are clearly visible (Fig. 2, *gb*, *st*, *pr*, *y*). In Fig. 3 the 116-hour-old embryo, dissected from a British Columbia host larva, is completely developed and ready to hatch. Figure 4 is of a 52-day-old encapsulated embryo dissected from a Manitoba host larva. Like the embryo in Fig. 2, it is in the late germ band stage. This type of immune reaction may happen during the egg or larval stage of parasitic insects within unsuitable hosts (1, 7, 21, 22, 23, 25); however, with *M. tenthredinis* it usually occurs between 70 and 80 hours after the egg is laid in host larvae from Manitoba, when the egg has developed to the late germ band stage.

The capsule is formed by an accumulation of basophilic host blood cells around the developing egg. They are round to oval in the earliest stages of capsule formation (Fig. 7, *o cl*). As their numbers increase about the embryo, those nearest to the chorion soon become flattened and appear spindle-shaped in sectioned material (Fig. 5, *f cl*). After treatment with malt diastase, PAS-positive inclusions were observed in these oval and 'spindle-shaped' cells. These cytoplasmic inclusions also stained beta (purple) metachromatically with toluidine blue at pH 5.0, but not at pH 2.6, before and after ribonuclease and hyaluronidase exposure. Such properties indicate that these inclusions contain some neutral mucopolysaccharide and (or) mucoprotein (17). Cytoplasmic mucopolysaccharide has been reported in the blood cells of insects: Rizki (20) noted the presence of cytoplasmic mucopolysaccharide in the 'plasmatocytes' of the fly *Drosophila melanogaster* Meig., and neutral mucopolysaccharide has been found in the 'basophilic granulocytes' of the beetle *Allomyrina dichotomus* (L.) (16) and in the 'amoebocytes' of the hemipteran *Rhodnius prolixus* Stål (28). Though histochemical testing of these inclusions in the blood cells of *P. erichsonii* was limited, they appear to be of a mucopolysaccharide nature.

FIGS. 1–4. *Mesoleius tenthredinis*. 1. Lateral view of a newly laid egg. 2. Lateral view of a 75-hour-old embryo dissected from a British Columbia larch sawfly larva. 3. Lateral view of a 116-hour-old embryo, completely developed and ready to hatch, dissected from a British Columbia larch sawfly larva. 4. Lateral view of a 52-day-old encapsulated embryo dissected from a Manitoba larch sawfly larva. Compare with Fig. 2.

ABBREVIATIONS (Figs. 1–8): *ant*, anterior; *cap*, capsule; *ch*, chorion; *f cl*, flat cell; *gb*, germ band; *gn rg*, gnathal region; *hc*, head capsule; *mes*, mesoderm; *mg*, mid-gut; *mi*, micropyle; *mn*, mandible; *o cl*, oval cell; *pc*, pole cells; *pr*, proctodaeum; *s lyr*, sheath layer; *ser*, serosa; *st*, stomodaeum; *vent*, ventral; *y*, yolk.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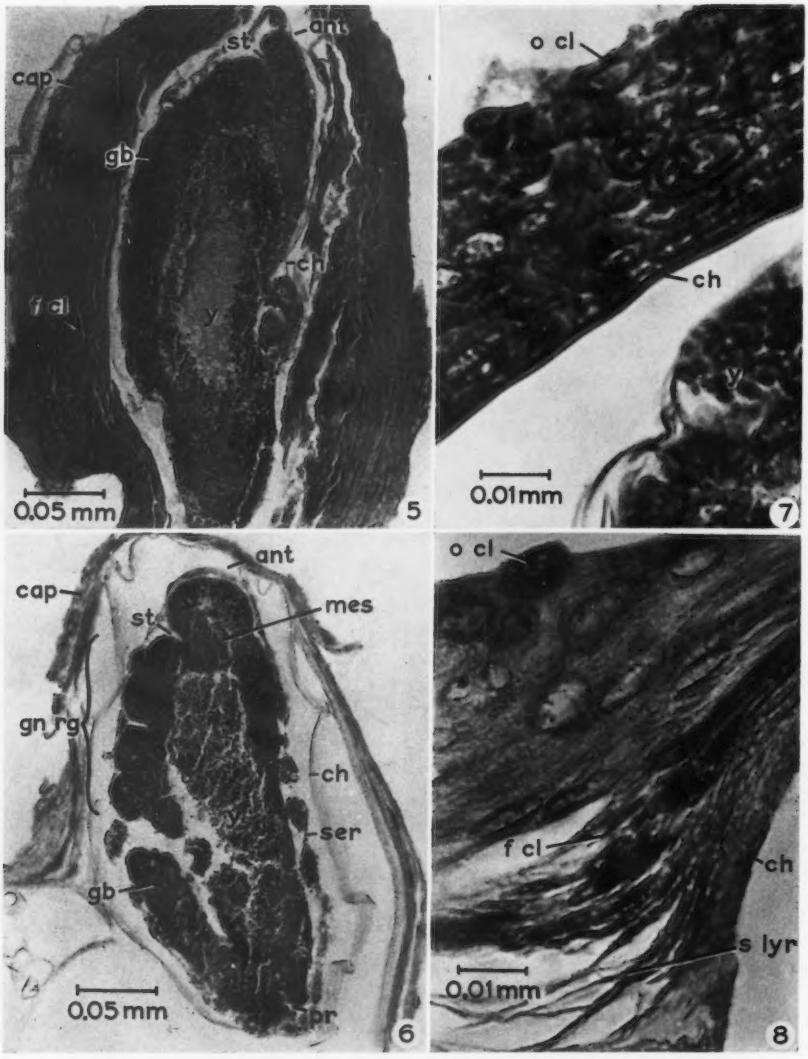

FIGS. 5–8. *M. tenthredinis*. 5. Sagittal section of a 7-day-old encapsulated embryo dissected from a Manitoba larch sawfly larva, showing the cellular nature of a recently formed capsule. 6. Sagittal section of one of three 24-day-old embryos encapsulated together dissected from a Manitoba larch sawfly larva, showing the cellular (outer) and sheath (inner) layers of the capsule. 7. Enlarged view of part of the capsule surrounding a 76-hour-old embryo. Cells at the inner edge of the capsule are beginning to flatten. 8. Enlarged view of part of the capsule surrounding a 24-day-old embryo, showing the cellular (outer) and sheath (inner) layers of the capsule.

The 'spindle-shaped' cells acquire a fibrous cytoplasm, their nuclei disappear, and eventually the cells become a mass of concentric sheaths of non-cellular material. The outer sheaths of the capsule are eosinophilic and remain PAS-positive after treatment with malt diastase. Toluidine blue at pH 5.0 only, before and after ribonuclease and hyaluronidase exposure, stains the sheaths in this region of the capsule greenish blue with beta metachromatic strands scattered throughout them. The inner sheaths of the capsule (those nearest to the chorion of the egg) are basophilic, PAS-positive after treatment with malt diastase, and remain beta metachromatic when stained with toluidine blue at pH 5.0 only after exposure to ribonuclease and hyaluronidase, which suggest their mucopolysaccharide nature. Wigglesworth (28) noted some neutral mucopolysaccharide in the cytoplasmic inclusions of the 'amoebocytes' and in the connective tissue of *R. prolixus*, and reported that these inclusions were precursors of the connective tissue sheaths.

The cellular region (outer) and sheath layers (inner) of the capsule surrounding a 24-day-old embryo are visible in Fig. 8. Melanin formation was rare in the capsules examined. Muldrew (13) noted that capsules in healthy host larvae did not darken until at least 1 month after parasitization, and in some cases were not melanized when left in the host for 7 months after parasitization. Salt (21, 22, 23), in his studies on the effect of various hosts on the eggs and larvae of the alien ichneumonid *Exidechthis canescens* (Grav.), observed that if they reacted at all, the reaction occurred in one or both of two ways: by a haemocytic reaction and by the deposition of melanin. In *P. erichsonii*, the initial immune reaction occurs by the former method and is much slower in its manifestation than in the majority of hosts to which Salt exposed *E. canescens*.

Lazarenko (8) and Lartschenko (7) considered the insect 'spindle-shaped' cells, which fused to a syncytium containing connective tissue fibers about foreign material, to be special mesenchymal cells from the tissue spaces, and not blood cells. Wigglesworth (27) in his studies on *Rhodnius prolixus* noted that the 'phagocytic amoebocyte', the commonest type of blood cell, was responsible for the formation of connective tissue sheath substances and the encapsulation of foreign particles with concentric sheaths of non-cellular material. This material was either secreted by the amoebocytes as droplets or strings of mucopolysaccharide, or the amoebocytes were drawn into filaments in which the mucopolysaccharide formed elongated deposits. There is no indication of blood cell secretion of the capsular sheaths in *P. erichsonii*, as they appear to be formed by the actual breakdown of the phagocytic blood cells (Fig. 8, *f cl*) in the cellular region of the capsule, whose cytoplasm has previously become fibrous.

It has been suggested (13, 26, 28) that the capsule inhibits the embryonic development of the parasitoid by asphyxiation. Recent studies (3) of the effect of oxygen on multiparasitism in the Mediterranean flour moth, *Anagasta kühniella* (Zell.), indicated the possible validity of this statement. Usually, in cases of multiparasitism with *A. kühniella*, the development of the older parasitoid was normal while that of the younger inhibited. However, when

multiparasitized host larvae were exposed to an excess supply of oxygen, the younger parasitoid not only survived, but used its well-developed mandibles to attack the older one. In a reduced supply of oxygen, development of all parasitic stages was retarded. Not only did the parasitic larvae take longer to hatch, but many did not develop beyond the first instar and later became partly encysted by host blood cells.

Summary and Conclusions

Larch sawfly larvae from Newfoundland and British Columbia are still susceptible to *M. tenthredinis* from British Columbia, but within host larvae from Manitoba, encapsulation of the parasitoid occurs when the embryo is in the late germ band stage. The capsule, formed by an accumulation of phagocytic host blood cells that contain cytoplasmic inclusions of mucopolysaccharide, inhibits further embryonic development of the parasitoid, possibly by interfering with its oxygen supply.

Acknowledgments

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ABNORMALITIES IN SECOND-STAGE LARVAE OF HETERODERA TRIFOLII GOFFART, 1932 (NEMATODA:HETERODERIDAE)[1]

ROLAND H. MULVEY

Abstract

Second-generation females of a cyst-forming nematode, *Heterodera trifolii* Goffart, 1932, from a mixed population of this species and the sugar-beet nematode, *H. schachtii* Schmidt, 1871, produced small, normal, giant, and abnormal second-stage larvae. The giant larvae averaged 670 μ in length, while the small larvae had an average body length of 404 μ. Abnormalities in the larvae occurred mostly in the tail development. It is suggested that the sperm of *H. schachtii* may have affected the normal chromosome complement of the *H. trifolii* oöcytes during oögenesis and was thereby responsible for the production of the different types of larvae recorded.

Introduction

Abnormalities in the head region of some second-stage larvae of two cyst-forming nematodes, *Heterodera schachtii* Schmidt, 1871, and *H. rostochiensis* Wollenweber, 1923, have been reported by Kampfe (3). Abnormalities in tail developments have as yet not been recorded.

The author (4) reported the occurrence of giant second-stage larvae in a population produced from second-generation females of the clover cyst nematode, *Heterodera trifolii* Goffart, 1932. These females originated from a mixed population of *H. schachtii* and *H. trifolii*. Further examination of the population in which the giant forms were found revealed the presence of both small and abnormally developed active second-stage larvae.

This report deals with the anatomical and relative size differences between normal, giant, small, and abnormal second-stage larvae of *H. trifolii*.

Materials and Methods

The method used to obtain the second-stage larvae was the same as that described by Mulvey (4). The active second-stage larvae were removed from the water in petri dishes, relaxed by heat, and subsequently mounted in lactophenol on glass slides. These were examined under the microscope and the dimensions were determined from camera lucida drawings of the specimens.

Anatomical Differences Observed

Abnormal Larvae

Abnormalities occurred mostly in the tail region. However, one specimen was found to be abnormally large in the area of the esophageal glands (Fig. 5), and the spear knobs of another were asymmetrically developed (Fig. 8, K).

Abnormalities in tail development ranged from double blunt tails (Figs. 7; 8, C, D) to tails with either very blunt or acute setoff termini (Figs. 6; 8, A, F, H), blunt terminus (Fig. 8, G), bluntly recurved (Figs. 4; 8, M), and

[1]Manuscript received April 27, 1960.
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

abnormally curved tails (Fig. 8, P). However, as shown in Fig. 8, the head area and stylet were generally normal.

Giant Larvae

Some variation was found both in the head and tail areas of several giant second-stage larvae. The tail of one specimen (Fig. 8, Q) had a very blunt terminus, and the spear knobs (Fig. 8, R) of the same specimen were large and pointed.

Small Larvae

These were fairly common in the population examined. As shown in Table I, the body length was on an average considerably less than that of the normal second-stage larvae. Franklin (1) reported the body length of normal second-stage larvae of *H. trifolii* within the range of 491–504 μ. Hirschmann (2) found the range to be 443–547 μ with a mean body length of 496 μ.

Dimensions

TABLE I

Measurements of various types of *Heterodera trifolii* second-stage larvae from the same population

Type of larvae	Number of larvae	Average length and range	
		Body (μ)	Stylet (μ)
Normal (Fig. 3)	30	495 (450–520)	27.0 (25–29)
Small (Fig. 2)	10	404 (370–440)	23.2 (21–28)
Giant (Fig. 1)	17	670 (610–760)	31.7 (30–35)
Abnormal (Figs. 4–6)	10	435 (370–500)	25.3 (22–28)

Discussion

The abnormalities in the second-stage larvae of this species encountered in this study present features of interest from the cytological standpoint. The male sperm of *H. schachtii* is apparently capable of effecting a change in the normal chromosome complement of *H. trifolii* oöcytes during oögenesis. These changes are apparently responsible for the production of the various types of larvae recorded.

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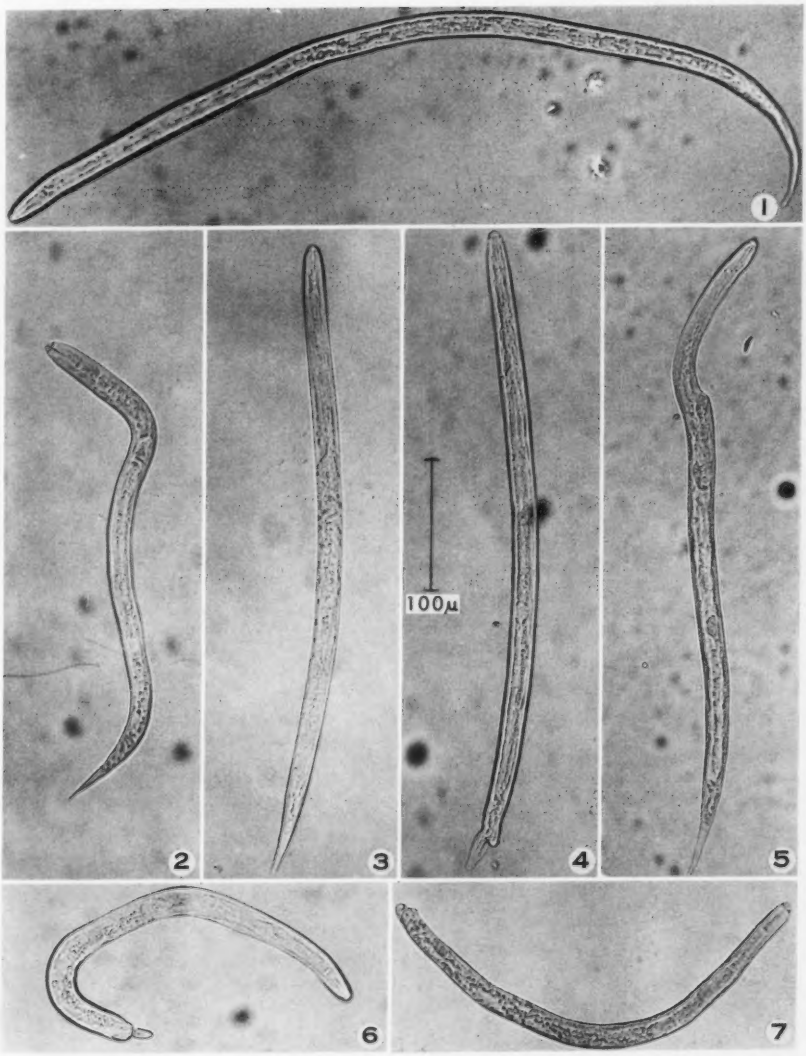
PLATE I



FIGS. 1–7. Photomicrographs of normal, small, giant, and abnormal second-stage larvae of *Heterodera trifolii*. All figures of the same magnification.

FIG. 1. Giant larva. FIG. 2. Small larva. FIG. 3. Normal larva showing acutely pointed tail. FIGS. 4, 6, and 7. Abnormal larvae showing variations in tail development and body length. FIG. 5. Abnormal larva showing abnormal enlargement of the body in the esophageal region.

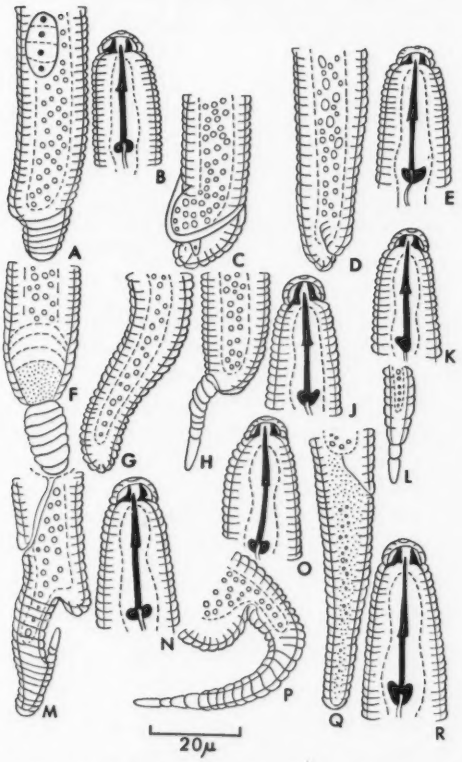


FIG. 8. Camera lucida drawings of heads and tails of various types of second-stage larvae of *H. trifolii*. All drawings of the same magnification.

A and B, head and tail of abnormal larva, the head is normal but the tail is blunt and underdeveloped; C, larva with double blunt tail and very small terminal process; D and E, larvae with normal head region and blunt double tail; F, larva with blunt, setoff terminus; G, larva with bluntly rounded tail; H and J, larva with acute, setoff tail terminus and normal head region; K and L, larva with underdeveloped stylet knob and short tail; M and N, larva with small acute tail terminus set off to dorsal side and with normal head region; O and P, larva with curved acute tail and normal head region; Q and R, giant larva showing very bluntly rounded tail terminus and long stout stylet shaft with large knobs.

MANISTRONGYLUS MEYERI (TRAVASSOS, 1937) GEN. NOV., AND NECATOR AMERICANUS FROM THE PANGOLIN[1]

THOMAS W. M. CAMERON AND BETTY J. MYERS

Abstract

Manistrongylus gen. nov. is proposed for the trichostrongyle previously described as *Trichoskrjabinia costata*. The present material was obtained from *Manis pentadactyla pentadactyla* from Formosa. *Necator americanus* is recorded from the same host.

In a collection of nematodes from the pangolin (*Manis pentadactyla pentadactyla*) collected in Formosa under the direction of Dr. Robert E. Kuntz (CDR, MSC,USN, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan) are examples of three species of nematodes. One of these is a larval ascarid which cannot be identified further. The other two are of considerable interest.

There are 30 specimens—15 males and 15 females—collected from six animals. These specimens are identical with a species identified in 1941 by Chin (5) as *Trichoskrjabinia costata* (Meyer, 1896). We believe that this species does not belong to this genus, which occurs in reptiles, and we have, accordingly, made it the type of a new genus, *Manistrongylus*, belonging to the family Trichostrongylidae.

Manistrongylus meyeri (Travassos, 1937)

Description

The slender females measure approximately 9 to 11 mm long and are loosely coiled in two or three whorls; the males are approximately 6 to 7 mm long with a single whorl. The cephalic cuticle is inflated and there is no buccal capsule. Cervical papillae are present, 0.33 mm from the cephalic end (Fig. 1). The oesophagus is claviform, 0.45 mm long in the females and 0.36 mm long in the males. The excretory pore opens in the females 0.42 mm, and in the males, 0.25 mm, from the anterior end.

Females.—The ovejector and uteri are opposed and the vulva opens about 2 mm from the tip of the tail. The ova are thin-shelled, measuring *in utero* 60 to 63 μ by 28 to 30 μ. The tail is 0.33 mm in length, bluntly rounded with a terminal spine (Fig. 2).

Males.—A pair of small prebursal papillae is present just anterior to the bursa. The bursa, which has a small dorsal lobe and an indentation between the externodorsal and the lateral rays, is ornamented with cuticular bosses. The arrangement and the relative size of the rays are shown in Fig. 3. The dorsal ray is bifurcate in its terminal third; each branch in turn is bifurcated, the external bifurcation being relatively massive and undulating, while the

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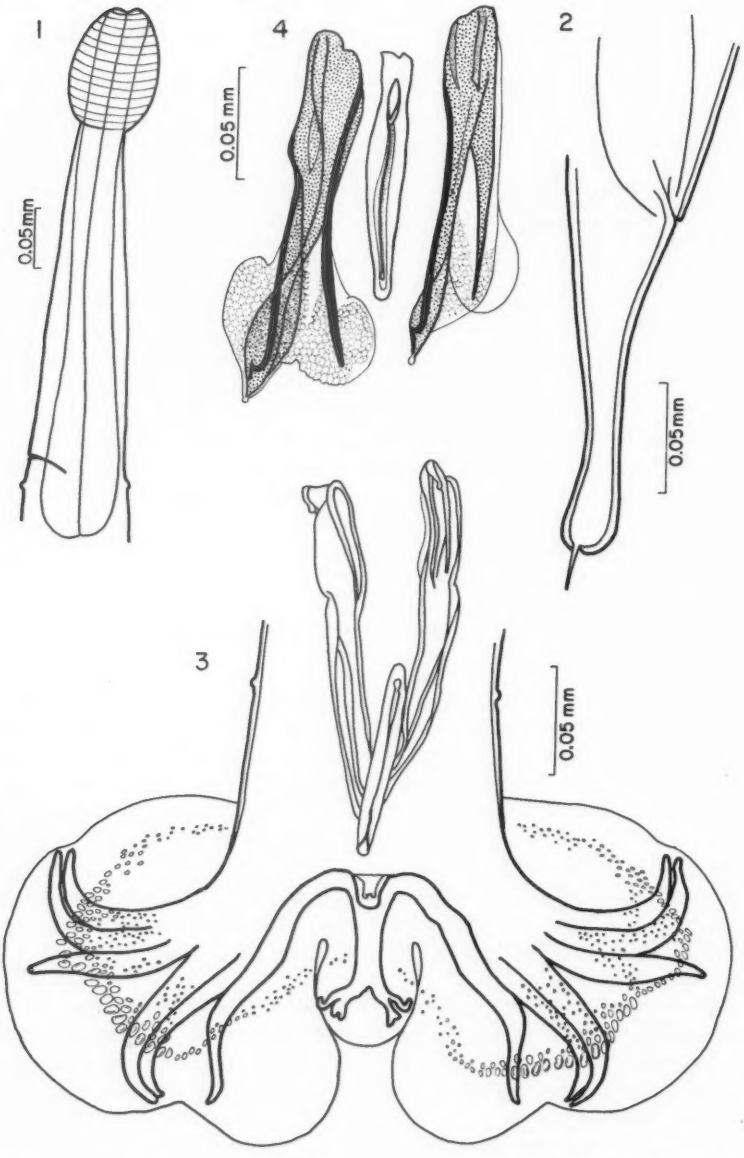
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.



FIGS. 1–4. *Manistrongylus meyeri* (Travassos, 1937) gen. nov. 1. Cephalic extremity. 2. Posterior extremity of female. 3. Male bursa viewed from ventral surface. 4. Spicules and gubernaculum.

dorsal branch terminates in two small digitations. The complete dorsal rays resemble the antlers of a deer. The externodorsal ray arises from the base of the main dorsal trunk and extends parallel with and close to the posterolateral ray for two-thirds of its length; its terminal third is sinuous and its tip is directed towards the ventral rays. The mediolateral and posterolateral rays are parallel to each other and separate only at their distal tips, which are directed dorsally; they terminate at the edge of the bursa. The externolateral ray is long and parallel to the lateroventral ray for about two-thirds of its length; it is almost straight and terminates a short distance from the edge of the bursa. The ventroventral and the lateroventral rays are parallel for their entire length and are directed anteriorly. The genital cone has two small papillae but there is no accessory bursal membrane. The gubernaculum is elongated and grooved, measuring 0.1 mm in length. The spicules are equal, 0.15 mm long; each is split into a thin tapering shaft and a thicker portion ending in a hook. A complex spicular membrane is present (Fig. 4).

Discussion

This species of trichostrongyle was described originally by Meyer (7) from this host under the name of *Strongylus costata*. Travassos (11) changed the name to *Trichostrongylus* (s.l.) *meyeri*, *Strongylus costata* being preoccupied. Chin (5) redescribed this species under the old specific name but referred it to the genus *Trichoskrjabinia* Travassos, 1937, which was established for the species *Oswaldocruzia malayana* Baylis, 1933 from a fresh-water tortoise. Examination of the description given by Baylis (3) for *O. malayana* shows that the species from the pangolin cannot be a member of this genus. It differs from it in the following particulars. There is no cephalic inflation or longitudinal ridges. The dorsal ray is without the long slender trunk and the complicated digitations of *Trichoskrjabinia*, the ventral rays are curved anteriorly, and the mediolateral and dorsolateral rays dorsally.

We have made it the type and only species of a new genus, *Manistrongylus*, characterized by the structure of the male bursa and by the complex spicular formation.

The type and only species is *Manistrongylus meyeri* (Travassos). Synonyms: *Trichoskrjabinia costata* (Meyer, 1896) Chin, 1941; *Trichostrongylus* (s.l.) *meyeri* Travassos, 1937; *Strongylus costata* Meyer, 1896 nec. Rudolphi, 1819.

The phylogenetic relationships of the pangolin have long been a matter of doubt. Earlier workers regarded this animal (as well as the aardvark) as belonging to the order Edentata. The Edentata are otherwise confined to South America where they occurred in large numbers during the entire Tertiary. While the group is highly specialized, it is generally believed that the Edentata arose from the protoinsectivores about the end of the Mesozoic or the beginning of the Paleocene. Modern workers have rejected the inclusion of the African aardvark among the Edentata and have had considerable doubts about the status of the pangolins, which had an apparently wide distribution in the Old World in the latter part of the Tertiary but are now confined to tropical Africa and Asia.

Simpson (9) divides the living members of the order Edentata into the infraorder Pilosa, including the sloths and anteaters, and the infraorder Cingulata, including the armadillos. He places the pangolins in a separate order—the Pholidata—distinct from the South American edentates, but states that it cannot be denied that the two "may have had a common ancestry, but this has not been demonstrated as a fact or even as a real probability, and if it be true, then the common ancestry must have been exceedingly remote (probably before the edentates were differentiated clearly from the proto-insectivores)".

It is accepted that South America was separated from the rest of the world from late in the Mesozoic until the Pliocene—with a possible junction with the Antilles late in the Tertiary. During the first half of the Tertiary, its fauna included marsupials, edentates, and some primitive ungulates of which only some opossums and a few edentates survive. About mid-Tertiary, fossils of platyrrhine primates and hystricomorph rodents appear. All other mammals entered the continent from North America late in the Pliocene or during the Pleistocene.

The Tertiary is believed to have lasted for some 60 million years and if the pangolins arose from the same original stock as the South American edentates, this postulates a separation of at least that time.

The trichostrongyle described in this paper is not incompatible with such an origin. Not only does it show quite close resemblances to those in true edentates, but it also suggests a Mesozoic origin of this group of nematodes and a relatively slow rate of evolution with a probably high degree of host specificity.

Necator americanus (Stiles, 1902)

In addition to this species there were 16 specimens of nematodes from three pangolins which agree with the description of *Necator americanus* given by Stiles (10), Looss (6), Ackert and Payne (1), and Baylis (4). Because of the unusual host and the presence of a few minor differences observed in the morphology a description of the buccal capsule and the male bursa is given.

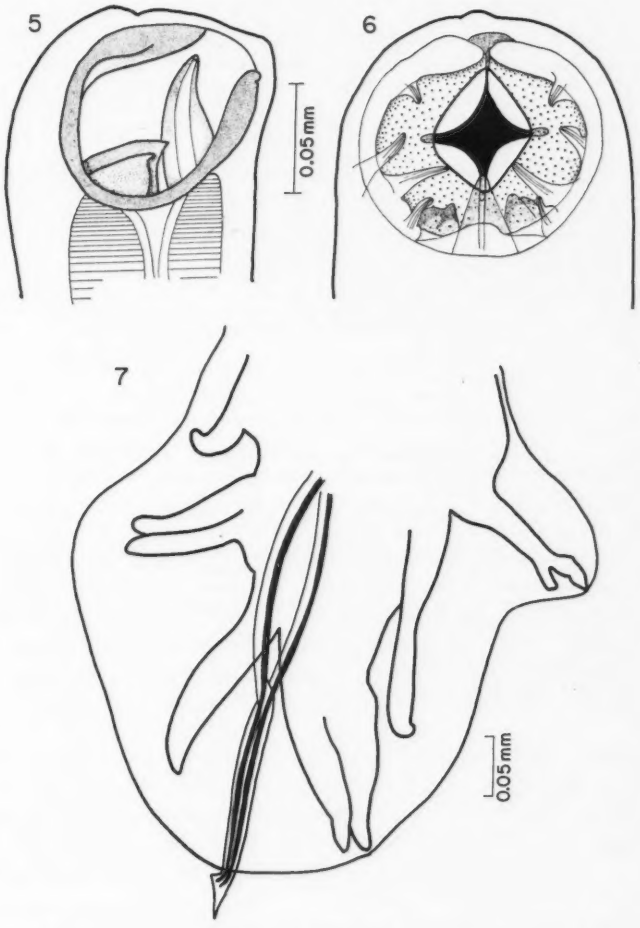
Buccal Cavity

The oral opening is trapezoidal. Both dorsal and ventral plates meet in the median line. The oral surface has eight papillae, four on each side; the dorsal lateral papillae extend to the proximal edge of the dorsal plate; the dorsal papillae arise near the dorsal lateral papillae but terminate midway to the dorsal plate. The dorsal cone is prominent, conical in dorsal aspect, and very long. Lateral lancets are well developed and cusp-shaped. The smaller ventral lancets are triangular and point towards the median line (Figs. 5 and 6).

Male Bursa

There are well-developed prebursal papillae which resemble rays. The bursa has two lateral lobes and a small dorsal one which is divided into two parts, each supported by the bifurcated dorsal ray. All rays are stout. The

ventral and the lateral rays are parallel. The externolateral ray arises from the main stem of the lateral ray; it does not reach the margin of the bursa. The posterolateral and the mediolateral rays are parallel for two-thirds of their length and terminate at the margin of the bursa. The externodorsal ray is thinner than the lateral rays. The dorsal ray is divided almost to its origin, each branch terminating in two digitations which are shaped rather like a shoe. The filiform spicules are united by the spicule membrane posteriorly and terminate in a barbed point, one spicule forming the barb and the other the distal end (Fig. 7).



FIGS. 5–7. *Necator americanus*. 5. Lateral view of mouth capsule. 6. Dorsal view of mouth capsule. 7. Lateral view of male bursa.

There are no other differences in size or other characteristics between specimens from man collected in China and the present material. Those differences noted above are comparatively small and there seems to be no reason to regard them as being more than intraspecific variations. These specimens from the pangolin are accordingly referred to *Necator americanus*. Previous records of this same species from pangolins have been made by Baylis (2), Sharma (8), and Wu and Hu (12).

As *Necator americanus* enters its host by penetration of the skin, there is no difficulty in understanding how pangolins can become infected. It is of considerable interest, however, to find that worms can become sexually mature in this host and the fact that three of the present animals were infected, combined with the previous records of three other workers, shows that this is not a case of occasional parasitism but that the pangolin must be considered as a normal host.

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SOME FURTHER RECORDS OF PELAGIC POLYCHAETA FROM THE NORTHEAST PACIFIC NORTH OF LATITUDE 40° N. AND EAST OF LONGITUDE 175° W., TOGETHER WITH RECORDS OF SIPHONOPHORA, MOLLUSCA, AND TUNICATA FROM THE SAME REGION[1]

E. Berkeley and C. Berkeley

Abstract

Seventeen species of pelagic Polychaeta from the northeastern Pacific are recorded. Of these, five (*Sphaerosyllis pirifera* Claparède, *Dorvillea kefersteini* McIntosh, *Spiophanes cirrata* Sars, *Magelona* sp., and *Flabelligera affinis* Sars) are species which, whilst not exclusively pelagic, are known to swim at some stage, particularly as larvae. One record (*Pedinosoma curtum* Reibisch) is new to the Pacific Ocean, two (*Pontodora pelagica* Greef and *Callizona nasuta* Greef) are new to the northeastern Pacific region, and four (*Pelagobia longicirrata* Greef, *Lopadorhynchus uncinatus* Fauvel, *Sagitella kowalewskii* Wagner, and *Callizona angelini* (Kinberg)) are records of northerly extensions of distribution. The first observation of a larval Cossura (probably of *C. longocirrata* Webster and Benedict) is recorded. *Poeobius meseres* Heath is recorded from a number of stations and the classification of Poeobiidae is discussed.

Eight species of Siphonophora are listed, all of which constitute new records for the region covered by the present collections. Six species of Mollusca, comprising five Pteropoda and one Heteropoda, are recorded, none of which have been identified previously in northeastern Pacific plankton. Three of the genera of Pteropoda (*Anopsia*, *Thliptodon*, and *Cliopsis*) are characteristic of warmer seas, the other two, and the heteropod, are widely distributed. Of the two species of Tunicata listed one is new to the northeastern Pacific.

Introduction

In 1957 (6) we recorded a number of pelagic polychaetes which had been collected in plankton samples by the vessels engaged in the survey activities of the Fisheries Research Board of Canada in the northeast Pacific during 1955 and 1956. The present paper records species taken in the course of similar activities during 1957, 1958, and 1959, together with notes on a few others which, though not usually regarded as pelagic, have been found free-swimming in the region in which the collections were made.* Several of the species mentioned herein were already recorded in 1957, but from single, or few, specimens. Others (such as *Tomopteris septentrionalis* Quatrefages and *Plotohelmis tenuis* (Apstein)), which are of very general occurrence, are now omitted.

During the years we have been examining specimens from these plankton collections we have encountered a number of animals belonging to groups other than the Polychaeta. These we have sent to specialists in the several fields for identification and we now append their findings in respect of the Siphonophora, Mollusca, and Tunicata.

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Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

*For chart of this region see reference 6.

Grateful acknowledgment is due to Mr. R. LeBrasseur, of the Fisheries Research Board of Canada, Nanaimo Biological Station, for the care exercised in the course of his sorting of the original plankton material, in separating the samples on which the identifications now recorded have been based.

Polychaeta

Phyllodocidae

Pelagobia longicirrata Greef. Fauvel (12), Ushakov (36)

47° 00′ N., 136° 07′ W., 150–0 m, one specimen
50° 07′ N., 144° 50′ W., 150–0 m, one specimen
50° 05′ N., 145° 20′ W., 150–0 m, one specimen
50° 01′ N., 145° 02′ W., 150–0 m, one specimen
43° 42′ N., 136° 00′ W., 300–200 m, four specimens
45° 02′ N., 147° 06′ W., 150–0 m, one specimen
50° 00′ N., 145° 00′ W., 150–0 m, several specimens

The present records extend the distribution of this species in the northeast Pacific northwards, that of Treadwell (35, as *P. vigiueri*), at 40° N., being the most northerly previous one. In the northwest Pacific Ushakov (37, map 1) indicates its occurrence at about 55° N. and he has also recorded it in the north polar region (38). It is widely distributed in the Atlantic, in the Mediterranean and Indian oceans, and in the Antarctic. It may, therefore, be regarded as cosmopolitan.

This species and *P. vigiueri* Gravier have been confused in the literature. They are now regarded as synonymous (37).

Pontodora pelagica Greef. Fauvel (12), Ushakov (37)

50° 00′ N., 145° 00′ W., 1250–0 m, five anterior, one posterior, one median portion

The specimens agree with Fauvel's (12) and Ushakov's (37) descriptions. The cirri are not easily distinguished and a few of them are lost in some of the specimens. The orange spotting (due, apparently, to an oily secretion) is very conspicuous in some of them.

The species has not been described previously from the northeast Pacific and there appears to be no record of it anywhere in the north Pacific north of about 40° N. (37). Ushakov (37) records several instances of its occurrence off the coast of Japan and from the Arctic Ocean between 75° N. and 85° N. (38). He considers *Epitoka pelagica* Treadwell is a synonym, a conclusion with which we agree. All other records from the Pacific are from the southern hemisphere (35). The species is known from the Mediterranean and, in the Atlantic, from the Canary Islands.

Lopadorhynchus uncinatus Fauvel. Fauvel (12), Wesenberg-Lund (39), Dales (11)

54° 30′ N., 152° 00′ W., 150–0 m, one specimen

A single complete specimen a little more than 1 mm long, probably larval, agrees completely with the characters of the adult as given by the above

authors. Dales' record from about 38° N. (11, Fig. 6) is the most northerly in the northeast Pacific hitherto. It is now recorded considerably further north. Treadwell (35, as *L. brevis* and *L. nans*) records it from off Japan and from numerous stations in the South Pacific. It is common in the Mediterranean and is recorded from the north and south Atlantic.

Pedinosoma curtum Reibisch. Reibisch (31), Fauvel (12)

53° 32′ N., 151° 57′ W., 150–0 m, one specimen

A single specimen 1 mm long agrees with the descriptions of Reibisch and Fauvel. The species has not been recorded previously from the Pacific. Earlier records are mainly from the southern Atlantic between the equator and 20° N., the most northerly point reached being in the neighborhood of the Azores (31, Taf. 7), and in the Mediterranean.

Phalocrophorus pictus Greef. Fauvel (12)

50° 28′ N., 131° 11′ W., 150–0 m, one specimen
43° 42′ N., 136° 00′ W., 150–0 m, two specimens, one with extended jaws
50° 01′ N., 145° 02′ W. (approximate), one specimen

Our previous record of this species (6) remains the farthest north in the north Pacific.

Typhloscolecidae

Travisiopsis lobifera Levinsen. Fauvel (12)

51° 00′ N., 130° 00′ W., 20-m tow, three large specimens
50° 00′ N., 145° 00′ W., 150–0 m, three specimens
47° 30′ N., 138° 54′ W., surface, one specimen

This species was recorded from a single specimen in 1957 (6). It is now found at about the same latitude, but further east.

Typhloscolex mülleri Busch. Fauvel (12)

50° 55′ N., 129° 08′ W. (approximate), 50-m tow, one specimen
50° 27′ N., 132° 11′ W., 150–0 m, one specimen
52° 02′ N., 136° 00′ W., 50-m tow, two specimens
55° 03′ N., 149° 54′ W., 150–0 m, one specimen
54° 30′ N., 152° 00′ W., 150–0 m, one specimen

In addition to the above the species has been taken from four other stations in the map area for which bearings are not available. It was reported in 1957 (6) from three stations and would thus appear to be fairly common.

Sagitella kowalewskii Wagner. Fauvel (12)

50° 01′ N., 145° 02′ W., 1200–0 m, one specimen
43° 42′ N., 136° 00′ W., 300–200 m, four specimens
50° 01′ N., 145° 02′ W., 150–0 m, one specimen

This species was recorded by one of us (E. B.) off the west coast of Vancouver Island at about 49° N. in 1930 (2). This remained the most northerly point in the northeast Pacific from which it was known until the present

records. It was, however, previously found at a slightly more northerly station in the northwest Pacific by Ushakov (37, map 4). It is more generally known from warmer seas.

Syllidae

Sphaerosyllis pirifera Claparède. Fauvel (12)

57° 32′ N., 151° 42′ W., 150–0 m, one specimen

This species, previously recorded from both coasts of Vancouver Island (3), has not, hitherto, been taken as a planktont. Fauvel (12) mentions swimming forms with long natatory setae in the male. These characterize the present specimen. It is interesting to find it so far from coastal waters.

Autolytus varius Treadwell. *Sacconereis*. E. and C. Berkeley (3)

53° 53′ N., 148° 00′ W., 150–0 m, four specimens

This species is a well-known offshore planktont in the Nanaimo region in the *Sacconereis* phase, but has not previously been found so far at sea. The specimens are typical.

Alciopidae

Callizona angelini (Kinberg). Fauvel (12)

50° 00′ N., 145° 00′ W., 150–0 m, two specimens
52° 30′ N., 142° 00′ W., surface at night, one specimen
53° 17′ N., 143° 53′ W., 25-m tow, one specimen
50° 00′ N., 145° 00′ W., 150–0 m, three specimens
55° 40′ N., 151° 10′ W., surface, one specimen

The earliest records of this species in the northeast Pacific were off the east coast of Vancouver Island at about 50° N. lat. and 125° W. long. (2). In 1957 (6) we listed a second one at about the same latitude, but as far west as 169° W. The present records are from stations intermediary in longitude between these extremes, thus extending the distribution across a considerable region of the width of the map area at about 50° N. lat. The northerly range is now extended to 55° N. lat.

The species is recorded off Japan and, widely, between 20° N. lat. and 20° S. lat. by Treadwell (35, as *Rhynchonerella pycnocera*). It is fairly common in the Atlantic (39).

Callizona nasuta Greef. Fauvel (12), Wesenberg-Lund (39)

48° 32′ N., 126° 09′ W., 50–0 m, one specimen
45° 00′ N., 147° 00′ W., 150–0 m, one anterior fragment
54° 30′ N., 152° 00′ W., 150–0 m, one anterior fragment

This species has not been recorded previously from the northeast Pacific. It is, however, known off the coast of Japan (37). The present specimens are typical and are readily recognizable even in the absence of the posterior regions. There is only one heavy and simple acicular seta in any of the anterior setigers. This, the projection of the central part of the prostomium in front of the eyes, and the narrow, sharply pointed, dorsal cirri are quite characteristic. It is known from the Atlantic and Mediterranean.

Tomopteridae

Tomopteris (*Johnstonella*) *renata*. E. and C. Berkeley (3)

50° 26′ N., 151° 24′ W., 150–0 m, one incomplete specimen
50° 01′ N., 145° 02′ W., 1200–0 m, one incomplete specimen
50° 01′ N., 145° 02′ W., 150–0 m, three good specimens
50° 01′ N., 145° 02′ W., 150–0 m, one specimen
55° 00′ N., 150° 00′ W., 60-m tow, one good specimen

In our 1957 paper (6) we commented on the absence of this species from the plankton samples and suggested that it might be an inshore form in the northeast Pacific, though it had been taken in open waters from regions farther west (36). The above records show that this surmise was erroneous. The characters differentiating the present species from *T. pacifica* Izuka, which we pointed out in our previous paper (6), are well exemplified in the present specimens. The last one listed is the largest yet known (45 mm long, excluding the tail).

Eunicidae

Dorvillea kefersteini McIntosh. McIntosh (27), Fauvel (12)

50° 55′ N., 129° 08′ W., 50–0 m, one specimen

The single small specimen (about 5 mm long as preserved) agrees with this species except in that the antennae are rather shorter and thicker than described (cf. ref. 12, Fig. 177 *m*). Ths may well be due to excessive contraction. The dorsal cirri lack both cirrophores and acicula, a character it shares with *D. gracilis* Hartman, but it differs from that species in having the palps non-articulate, except at the tips, and parapodia with much longer setal lobes.

D. kefersteini is not a true pelagic species, though McIntosh (27) records its ability to swim. It has not been recorded previously from the west coast of North America.

Spionidae

Spiophanes cirrata Sars. E. and C. Berkeley (4)

48° 33′ N., 123° 30′ W., surface, eight larval specimens

The specimens vary in length from 1 to 2 mm. The longer individuals are at an advanced larval stage consisting of 20 segments. Most of the swimming setae have been shed. These larvae are readily differentiated from those of *S. bombyx* (Claparède), the only other, and much less common, species known in the region, by the absence of prostomial "horns" and the presence of tridentate crotchets in posterior setigers.

The adults of this species are mud dwellers. The larvae have not been described, but a very complete account of those of a very nearly allied form, *S. kröyeri* Grube, is given by Hannerz (15). The present specimens agree closely with this account. It would not be anticipated that the single character differentiating *S. cirrata* from *S. kröyeri* (the presence of "soies bacillaires" in the former, but not in the latter) would be apparent in the larvae. Larvae of *S. kröyeri* are said to be common at particular seasons in the plankton in Gullmar Fjord, Sweden (15).

Magelonidae

Magelona sp. E. and C. Berkeley (4)

44° 9′ N., 130° 53′ W., 100–0 m, four larvae

51° 34′ N., 133° 24′ W., 50–0 m, one larva

Most of the specimens are less than 2 mm long. Whilst their generic identity can be recognized from the long papillated palps the adult characters are not sufficiently developed for specific determination. Adults of two species, *M. japonica* Okuda and *M. pitelkai* Hartman, are known from the region. Assuming the larvae to belong to one or other of those species, *M. pitelkai* seems to be the more probable, judging by the form of the prostomium and of the special setae of the ninth setiger. The larvae of neither *M. japonica* nor *M. pitelkai* have been recorded. Those of other species of *Magelona* are known to occur in the plankton (33, 22).

Cirratulidae

Cossura sp. (probably *C. longocirrata* Webster and Benedict). E. and C. Berkeley (5)

51° 34′ N., 133° 24′ W., 50–0 m, one larva

The specimen is about 2 mm long and consists of about 22 segments. Measurements are difficult owing to its curled condition. It is incomplete posteriorly. The single long median cirrus (branchia) is characteristic of the genus, but specific identification is impossible. It is probably *C. longocirrata* Webster and Benedict, since this is the only Cossura recorded from the northwest coast of North America north of California (5). Two other species are known from California, *C. candida* Hartman and *C. pygodactylata* Jones. The former is excluded in the present instance since the branchial process is attached to the second, instead of the third, setiger; the latter, however, remains a possibility (18). No setae other than very long fine larval capillaries can be detected. We have been unable to find any previous record of a larval Cossura.

Chloraemidae

Flabelligera affinis Sars. Fauvel (13)

Friday Harbour, Wash., U.S.A., swarming at dock

Five specimens, the longest of which measures 20 mm, were taken at a night light by Dr. R. L. Fernald of the Friday Harbour Laboratories. They were sexually mature and spawning. The only reference we have been able to trace to the swarming of this species is given by McIntosh (28) as having been observed by Dr. H. C. Sorby in the River Orwell, in England. Its occurrence at Friday Harbour (which falls within the region dealt with in this paper) seems noteworthy.

There is no previous record of the occurrence of the species at Friday Harbour nor elsewhere on the northwest coast of North America, *F. infundibuliformis* (Johnson) being the only representative of the genus known hitherto in the region. The present specimens differ from *F. infundibuliformis*, and

agree more closely with *F. affinis*, in respect of the much smaller number of segments, less sharply curved hooks, and the possession of two types of stalked cuticular papillae. The two species are, however, very closely allied. Pettibone (29) regards them as synonymous.

Miscellaneous Larvae

In addition to the above many larvae too small or imperfect to identify to species occur in the material. Amongst these, Spionidae are the most frequent, many of them sufficiently advanced to place in the genus *Polydora* by the presence of the modified fifth setiger. Eunicidae (Dorvillea?) and several kinds of Polynoidae also occur. The distribution seems to be indiscriminate.

Poeobiidae

Poeobius meseres Heath. Heath (17), Pickford (29)

53° 13′ N., 160° 53′ W., 400–200 m, several
53° 49′ N., 159° 32′ W., 300–200 m, several
54° 17′ N., 146° 37′ W., 400–300 m, several
51° 51′ N., 146° 37′ W., 400–300 m, several
51° 48′ N., 153° 20′ W., 400–300 m, several
54° 26′ N., 158° 07′ W., 400–300 m, several
48° 20′ N., 175° 30′ W., 300–200 m, several
48° 10′ N., 171° 21′ W., 200–100 m, several
52° 01′ N., 169° 44′ W., 400–300 m, several
49° 19′ N., 175° 30′ W., 200–100 m, several
51° 43′ N., 155° 00′ W., 150-m tow, several
53° 33′ N., 148° 00′ W., 150-m tow, several

The first observations of this species in our plankton material were made in 1956. It was not, however, reported in 1957 (6) since its identity had not then been determined. All, except the last two, of the above records date from 1956 when it occurred plentifully between long. 146° N. and long. 175° N. in the vicinity of 50° lat. The last two records date from 1958; since that time it has been found frequently in samples from 150 m or deeper. The species was originally recorded in 1930 from Monterey Bay, California (17), and subsequently in the same locality and elsewhere off the coast of California (16). At about the same time it was taken off SE. Alaska (30). Since then it has been collected by a number of expeditions exploring the north Pacific. McGowan (26) summarizes the localities (Fig. 3) and indicates a lack of data on distribution in the region of the area covered in the present paper. It has always been taken at moderate to deep levels. Most of the examples we have examined have been in the contracted condition figured by Heath (17, Plate 1, Fig. 1); only a few had the ends of the palps and tentacles emerging. Through the kindness of Dr. O. Hartman we have, however, been able to examine specimens collected off northern California by Dr. Martin Johnson which show the anterior region fully emerged and expanded as shown in her figure (16, Plate 1, Fig. 6). The length of our specimens varied from 6 to 17 mm.

The genus *Poeobius*, with type species *P. meseres*, was described from material collected in Monterey Bay, California, by Heath (17) and was regarded as a member of a new family Poeobiidae, to be included, provisionally, in the Echiuroidea. It was, however, recognized that it had certain affinities with the Polychaeta. Classification with the Echiuroidea was accepted by later workers until disputed by Fisher (14), who stressed the characters in which it differs from the Echiuroidea and agrees with the Polychaeta, but, in the absence of several characters hitherto regarded as essential to Polychaeta, found difficulty in including it in that group either. He suggested the setting up of a new phylum Poeobioidea to accommodate it. Pickford (30) whilst agreeing with Fisher as to its exclusion from Echiuroidea considered that, in the light of such affinities with Polychaeta as Heath and Fisher had pointed out and of others she was able to indicate, classification in that group was justified and concluded that it is an "aberrant polychaete". We are inclined to share Fisher's difficulty in including it in the Polychaeta in the complete absence of paired appendages, somatic segmentation, and setae.

Hartman (16) accepted Pickford's view and carried the argument in favor of polychaete analogy a stage further, suggesting its classification with the Flabelligeridae, but, in spite of the characters common to that family and *Poeobius*, which she indicated, the analogy is not altogether satisfying, in particular with regard to the comparison which is made between the gelatinous sheaths which occur in both. These differ fundamentally. That in *Poeobius* is internal, occupies a well-defined area between the hypodermis and the somatic musculature, and is sufficiently rigid to give the body in life considerable turgidity (17, p. 228). In *Flabelligera* the jelly is external, mucous, and follows only roughly the contour of the animal to form a protective covering.

On the whole there seems to be a case for classifying *Poeobius* amongst the Archiannelida, a group lending itself to the reception of such anomalous Annelida, until something is known of its development.

Siphonophora

Identification of the Siphonophora was kindly undertaken by Dr. G. O. Mackie, of the University of Alberta, who had already collected and identified some of the species recorded from localities in the region covered by the present paper. At his request these records are included here. They are indicated under each species by his initials or by those of the following collectors who sent him material: Mr. H. Menkes, of the Canadian Hydrographic Survey, Mr. A. Fontaine, of Victoria College, Victoria, B.C., and Dr. C. Carl, of the Provincial Museum, Victoria, B.C. Dr. Mackie wishes to acknowledge help he has received in the identifications from Mr. A. K. Totton of the British Museum.

Calcyophorae

Vogtia sp. Kölliker. Bigelow (9), Moser (25, Tafs. 17 and 18)
50° 00′ N., 145° 00′ W., 1200–0 m, one specimen, fragmentary

V. pentacantha Kölliker occurs in the Bering Sea and northwestern Pacific (9). It is characteristically a deep-sea form (25).

Dimophyes arctica Chun. Moser (25, Taf. 26)

50° 00′ N., 145° 00′ W., 150–0 m, one anterior nectophore

55° 58′ N., 150° 46′ W., 10–0 m, three anterior, one posterior nectophore (H. M.)

50° 00′ N., 145° 00′ W., 1200–0 m, one anterior nectophore

Collected in Bering Sea by U.S. Fisheries Steamer "Albatross" (9).

Muggiaea atlantica Cunningham. Moser (25, Taf. 1)

Ladysmith Harbour, V.I., 10-m tow, five complete polygastric stages and eight mature eudoxids (G.O.M.)

Friday Harbour, Wash., U.S.A. From float at night light, six polygastric specimens (G.O.M.)

A cosmopolitan species in little-contaminated coastal waters. Recorded from Monterey Bay, Cal., U.S.A. (10).

Physonectae

Agalma elegans Sars. Kawamura (19, unnumbered plate, Fig. 11), Totton (34, Fig. 24)

53° 30′ N., 138° 53′ W., 150–0 m, 29 adults, 16 larvae

52° 08′ N., 133° 26′ W., 20-m tow, one adult

48° 00′ N., 133° 12′ W., 150–0 m, seven larvae

53° 28′ N., 133° 32′ W., 150–0 m, five larvae

55° 00′ N., 150° 00′ W., 21–0 m, five larvae (H. M.)

55° 00′ N., 155° 00′ W., surface, five larvae (H. M.)

Occurs at Misaki, Japan (19). Not previously recorded from northeast Pacific.

Forskalia sp. Kölliker. Bedot (1), Totton (34, Figs. 29, 30, and 31)

54° 15′ N., 134° 40′ W., 100–0 m, one juvenile

This genus has not been recorded previously from the northeast Pacific.

Apolemia uvaria Lamarck. Moser (24, Fig. 507), Bigelow (8)

52° 02′ N., 136° 00′ W., 150–0 m, three specimens

This monotypic genus has not been recorded previously from the north Pacific.

Nanomia bijuga Delle Chiaje. Bigelow (8), Kawamura (19), Totton (34, Fig. 19D)

52° 00′ N., 127° W., 150–0 m, one nectophore

Oak Bay, Victoria, B.C., surface, two specimens (A. F.)

Friday Harbour, Wash., U.S.A. From below the wharf at night light. Twelve specimens, the largest with eight nectophores, the smallest with three. Luminescent, the luminescence appearing to correspond with the distribution of the reddish pigment (G.O.M.).

Occurs at Misaki, Japan (19). Not previously recorded from northeast Pacific.

Nanomia sp. (? *N. cara* Agassiz). Totton (34, Fig. 19A–C)
Oak Bay, Victoria, B.C., surface, two specimens (A. F.)
Between Sidney Island and Forest Island, B.C., one specimen (C. C.)
There is no previous record of *N. cara* from the north Pacific.

Mollusca

Prof. J. E. Morton, of the University of Auckland, New Zealand, was kind enough to classify the Mollusca in the collection and to him we extend cordial thanks. The quotations in the list which follows are from his notes. None of the species identified have been recorded previously from the northeastern Pacific.

HETEROPODA

Atlanta sp. Thiele (32)
51° 51′ N., 132° 00′ W., 150–0 m, one specimen
A second specimen, for which bearings are not available, "apparently of the same species" is in the collection. "The species is probably not the Pacific *fusca*, recorded at least as far north as San Francisco, but too small (only 1.5 mm) to fit this specimen. I should assign it to *A. gaudichaudi*, very widespread in the Pacific and relatively much more abundant there than the more dominant Atlantic species *A. peroni*."

PTEROPODA

THECOSOMATA

Euclio pyramidata Linné. Thiele (32, as *Clio*)
52° 18′ N., 134° 46′ W., 150–0 m, one specimen
50° 08′ N., 141° 02′ W., 150–0 m, one specimen

A third specimen, the bearings for which are not available, clearly belongs to this species. "The lateral processes are only faintly marked, but the lateral edges are characteristically distinct. The strong median dorsal rib is also clearly diagnostic." Two of the specimens are only sufficiently well preserved to be identified to genus with certainty, but "probably, on geographical grounds, the common species *pyramidata*".

GYMNOSOMATA

Anopsia sp. Thiele (32)
53° 58′ N., 142° 22′ W., 150–0 m, one specimen
"Specimen in preserved state shell-less, and not easily determinable, though with a well marked toothed thecosome gizzard, upon dissection. With the transverse furrows marking the body, and the small glandular nodules in the loose skin, it has some of the characters of this problematic form."

Notobranchaea macdonaldi Pelseneer. Thiele (32)

52° 27′ N., 167° 21′ W., 150–0 m, one specimen

"Body shape much distorted and head and foot lobes alone give any clue as to the identity. Hook sacs, however, reveal the expected cluster of short wide hooks, and from what one can still detect there is a fair agreement with Notobranchaea. The Pacific distribution of this genus, however, is highly uncertain."

Thliptodon sp. (probably *diaphanus* Meisenheimer). Morton (23)

53° 23′ N., 133° 45′ W., 300–200 m, one specimen

Two further specimens, for which bearings are not available, are attributed to this genus. Characterized by "the general expansion of the head, the loosely fitting and very roomy "skin" surrounding the viscera and particularly the very roomy hook sacs, with a sheaf of rather golden-coloured long hooks". In one case "the everted hook sacs make it a fine specimen and give a good identification of character, with the golden chitinous appearance of the hook sheaves". "Of the Thliptodon spp. *diaphanus* is the best known of the Pacific ones, probably the only one, and occurs in the upper layers as well as the deeper water."

Cliopsis krohni Troschel. Massy (20), Thiele (32)

58° 29′ N., 140° 51′ W., 150–0 m, one specimen

Characterized by "the very small visceral nucleus inside a spacious gelatinous "skin" with small whitish glandular nodules, and a very long proboscis invaginated or coiled within. Also the very far forward position of the swimming wings".

Tunicata

Dr. Leo Berner, of the Scripps Institute of Oceanography, La Jolla, Cal., very kindly identified the following two species of pelagic Tunicata in material submitted to him. All were in the southern region of the area fairly close in shore.

DOLIOLIDA

Doliolum (*Dolioletta*) *gegenbauri* Ulianin. Berrill (7)

44° 00′ N., 127° 02′ W., 150–0 m

42° 05′ N., 127° 35′ W., 150–0 m

46° 08′ N., 127° 01′ W., 150–0 m

This species is widely distributed in the Atlantic, but, in the Pacific, its previously recorded distribution seems to have been confined to the more southerly region.

SALPIDA

Salpa fusiformis Cuvier. Berrill (7)

44° 00′ N., 127° 00′ W. (approximate), surface

42° 05′ N., 127° 35′ W., 150–0 m

Dixon Entrance, oblique tow, 100–0 m

This species is known from the Pacific coast of North America from California to Bering Sea (21).

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ON THE DEVELOPMENT AND TRANSMISSION OF COSMOCERCOIDES DUKAE OF TERRESTRIAL MOLLUSCS IN ONTARIO[1]

ROY C. ANDERSON

Abstract

Nematodes from the mantle cavity of *Discus cronkhitei*, *Zonitoides arborea*, and *Deroceras gracile*, and the rectum of *Rana* spp., *Bufo americanus*, and *Ambystoma jeffersonianum* in Ontario are referred tentatively to *Cosmocercoides dukae* (Holl, 1928). In molluscs, females deposit third-stage larvae that leave the mantle cavity and appear in slime trails left by snails; when placed on the foot of snails these larvae entered the respiratory pore and went to the mantle cavity where they developed. Larvae were found in the genital tract and ovary of slugs and in eggs of both snails and slugs. These penetrated into the mantle cavity of embryo molluscs which subsequently hatched. All stages of *C. dukae* can survive for long periods in tap water or invertebrate saline. Females sometimes laid eggs which hatched if they contained a first-stage larva and the latter developed to the infective stage. Worms of all stages from molluscs fed to frogs were passed at intervals in the faeces and there was no evidence this species could establish itself in amphibians; the occurrence of cosmocercids in amphibians in Ontario is possibly the result of the latter eating infected molluscs, the worms being able to survive passage through the gut. The larvae and adults are described in detail herein.

Introduction

Deroceras gracile Rafinesque, *Discus cronkhitei* (Newcombe), and *Zonitoides arborea* (Say) in Ontario are frequently infected with a species of *Cosmocercoides*. In Algonquin Park near the Wildlife Research Station of the Ontario Department of Lands and Forests, the mantle cavities of almost 70% of *D. cronkhitei* and *Z. arborea* dissected in 1959 were infected. During the winter of 1959–60 we were surprised to find that about a third of the newly laid, transparent eggs of six *D. gracile* kept in a laboratory culture with *D. cronkhitei* and *Z. arborea* contained third-stage larvae of *Cosmocercoides*. Similar larvae were subsequently found in snail eggs. Few nematodes are known to be transmitted through eggs of their hosts and a study was commenced to determine the significance of these observations.

A review of the literature revealed we were not the first to observe nematode larvae in eggs of molluscs. Barthélemy (2) as early as 1858 noted larvae (one to four individuals per egg) in eggs of grey slugs ("limace grise") from his garden at Toulouse. They had attenuated tails and were said to be never more than a quarter of a millimeter in length. He named them *Ascaroides limacis*. His illustrations show he was probably dealing with a species of Cosmocercidae. Noting that newly laid eggs were frequently infected, Barthélemy thought the egg capsule was too tough for nematodes to penetrate and reasoned that they must enter the eggs in the genital tract of the slug. He dissected several slugs and found similar larvae in the ovary of two of them. Barthélemy believed

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the nematodes killed the developing embryo and reached adulthood in the debris of the egg. Larvae ingested with the food of the slug subsequently penetrated into the ovary where they became incorporated into the egg. He recognized deficiencies in his account of the life cycle.

Conte and Bonnet (7) observed larval and adult nematodes in the genital tract (especially the oviduct and seminal receptacles) of *Helix aspersa* from an area south of Lyon. Each slug contained 20–30 nematodes which they named *Angiostoma helicis*. Although they gave no illustration, the description agrees well with that of the Cosmocercidae. Their species was viviparous and Conte and Bonnet believed transmission of larvae took place during copulation.

Chitwood and Chitwood (5) noted *Cosmocercoides* in the intestine (*sic*) of *Opeas goodalli* and *Deroceras agreste* from greenhouses of the United States Department of Agriculture. They observed larvae of *Cosmocercoides* in eggs of *Deroceras agreste* but did not speculate on their possible significance.

Ogren (11) found *Cosmocercoides* in the mantle cavity of *Ashmunella rhyssa edentata*, *Polygyra fosteri*, *Retinella* sp., and *Deroceras* sp. from Illinois and New Mexico and he identified these nematodes as *Cosmocercoides dukae* (Holl, 1928), originally described from the colon and intestine of *Triturus viridescens* of Durham, North Carolina (9). Moreover, he placed as a synonym of *C. dukae*, *Cosmocercoides variabilis* (Harwood, 1930), originally described from various amphibians and reptiles from Texas (8). Later Ogren (12) reported *C. dukae* in *Deroceras gracile* from Wisconsin, Tennessee, and Illinois.

Ogren's (11) observations on the life cycle of *C. dukae* in snails were briefly as follows: Filiform larvae (which are deposited by the female), as well as adults and subadults, leave the mantle cavity and can be found in slime trails left by snails. Larvae (presumably the filiform larvae) will enter uninfected snails probably by working their way between the mantle and shell or via the respiratory pore. *Ashmunella rhyssa edentata* and *Polygyra fosteri* were said to have been experimentally infected but no details were given.

Chabaud and Brygoo (4), noting some apparently important differences in the accounts of the life cycles given by Harwood (8) and Ogren (11), questioned the validity of placing *Cosmocercoides variabilis* as a synonym of *C. dukae*. As noted above, Ogren claimed the nematodes in molluscs were viviparous and that larvae left infected snails and penetrated in some way into uninfected snails. Harwood, on the other hand, claimed his species from amphibians and reptiles was oviparous, the eggs developing to the infective stage in the external environment. Chabaud and Brygoo suggested, therefore, that there might be two varieties, one viviparous in molluscs and the other oviparous in amphibians. They preferred to call the nematodes in molluscs, *C. dukae* and the other *C. variabilis*. Unfortunately, *C. dukae* was originally also described from an amphibian, although its description is most summary, and its life cycle was not studied.

Besides describing the larvae and adults in detail we shall attempt to show in this article that (1) the nematodes in slugs and snails in Ontario are conspecific; (2) the life cycle can be completed in at least two ways, viz. infective larvae appearing in the slime trails of infected molluscs are able to infect other

molluscs that come in contact with them or infective larvae get into the genital tract and continue their development and when eggs are produced the larvae are incorporated into them and finally penetrate into the mantle cavity of the developing embryo; (3) in Ontario, at least, *Cosmocercoides* found in the rectum of amphibians seem identical with those in molluscs. Amphibians probably become infected from eating the latter, the worms being able to survive indefinitely in a wide range of environmental conditions, including the amphibian rectum.

We are reluctant to conclude definitely that the species in Ontario molluscs belongs to either *C. dukae* or *C. variabilis*. This question cannot be settled in a study restricted to fauna in Ontario. Harwood's observations need to be confirmed and future work will have to establish the validity of *Ascaroides limacis* Barthélemy and *Angiostoma helicis* Conte and Bonnet with the possibility that these European species are conspecific with our North American representatives. Many European molluscs (e.g. *Deroceras reticulatum* (Müll.) = *Limax agrestis* L.) now have a wide distribution in North America, including Ontario, and they may have introduced species of *Cosmocercoides* to native land molluscs. For these reasons the species studied in Ontario is only tentatively referred to *Cosmocercoides dukae* (Holl, 1928).

Materials and Methods

Snails, slugs, and amphibians collected in Algonquin Park and the Caledon Hills during August and September 1959 were maintained in the laboratory over winter. Frogs were kept at refrigeration temperatures or in cold running water (approximately 10–15° C). Snails and slugs, placed in large aquaria with sterilized leaf litter, were kept at about 19° C and fed lettuce and rolled oats. *Discus cronkhitei* and *Deroceras gracile* laid eggs in December. These were collected and placed on filter paper in stender dishes and kept at about 16° C.

Infective larvae were collected from female worms from slugs and placed on the feet of young snails placed in small drops of water. Observation usually determined whether the larvae had entered or at least become enmeshed in slime between the shell and the foot. These snails were then placed on filter paper in stender dishes, kept at 19° C, and fed lettuce, rolled oats, and calcium carbonate.

Larvae were studied after they had been killed by gentle heat and mounted under vaseline-ringed cover slips. *En face* preparations were made following a technique previously described (1).

Results and Conclusions

Morphology of the Larval Stages of Cosmocercoides dukae

General Considerations

Development of the first two larval stages of *C. dukae* usually takes place in the uteri of the female worm, the molted cuticle being expelled through the vulva. Generally, therefore, to locate these stages it was necessary to find and dissect females in which they were present; often only segmented eggs and

sometimes only infective larvae (third-stage) were present. It was, however, not always necessary to kill the females to obtain these larvae. The ovipositing process was highly unstable and many females removed from the mantle cavity and placed in tap water or invertebrate saline discharged the contents of their uteri regardless of the level of development of eggs or larvae. Segmented eggs discharged in this way died but those containing first-stage larvae hatched in the water and the larvae developed to the infective stage. Thus it was possible to follow the development from the first to the infective stage in specimens mounted in water under vaseline-ringed cover slips. It is worth mentioning that female worms that had discharged the contents of their uteri did not necessarily die; many lived up to 2 weeks under vaseline-ringed cover slips and even longer in water in dishes kept at lower temperatures (10–15° C).

The Egg (*Figs. 2–3*)

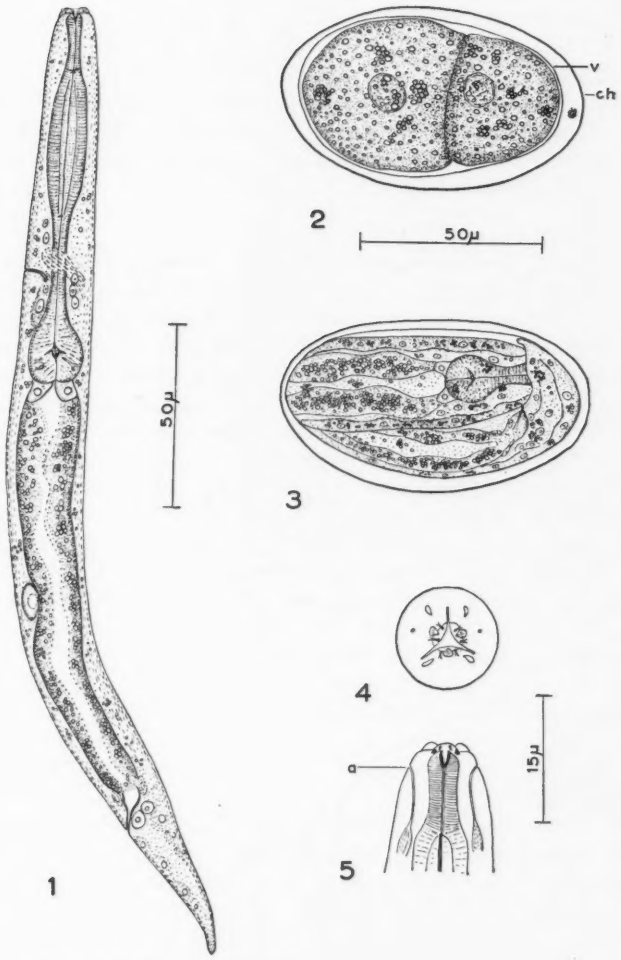
The oval-shaped egg, about 80 μ long and 50 μ wide, was composed of two capsules, a delicate, inner, vitelline membrane and an almost equally delicate chorion. A small body of protoplasm-like material often present in the perivitelline space, and sometimes between the chorion and vitelline membrane, may have represented a polar body.

The First-stage Larva (*Figs. 1, 4, 5*)

A first-stage larva hatched from an egg deposited 17 hours previously, had the following dimensions: Length 270 μ. Maximum width 22 μ, near the middle of the body. Oesophagus 102 μ in length consisting of a short, narrow, anterior part 18 μ in length in front of the opening of the dorsal oesophageal gland, a corpus 40 μ in length terminated posteriorly by the openings of the ventrolateral oesophageal glands, an isthmus 26 μ in length, and a bulb 18 μ in length and 14 μ in width. Nerve ring 69 μ and excretory pore 73 μ from the cephalic extremity. Single-celled genital primordium 162 μ from the head end. Tail 41 μ in length. Phasmids minute, about 30 μ from the caudal extremity.

The anterior extremity had three minute lips which did not contain arcadial tissue, with the result that they were transparent and easily distinguished from the rest of the body. The papillae were exceedingly minute and our interpretation, based on studies of lateral and *en face* views, may not be wholly accurate. Each lip seemed to carry a pair of internal papillae the nerve tracts of which could be seen passing through the lips. In addition, at least four flat, inconspicuous papillae seemed to be present at the bases of the lips. The amphids opened about 2 μ behind the bases of the lips; they led into a narrow canal about 7–8 μ in length which finally expanded into the amphidial pouch.

The anterior end of the oesophagus consisted of three lobes and its lumen in this region was broad and funnel-shaped in lateral view. In *en face* view the apices of the oesophageal lobes gave the appearance of three minute teeth. The terminal excretory duct was well defined and its distal part lay at right angles to the longitudinal axis of the body. The oesophagointestinal valve was highly developed. The intestine contained numerous refractory granules.



FIGS. 1–5. *Cosmocercoides dukae.*
FIG. 1. First-stage larva, lateral view.
FIG. 2. Egg in two-cell stage.
FIG. 3. Egg with first-stage larva.
FIG. 4. Anterior extremity of first-stage larva, *en face* view.
FIG. 5. Anterior extremity of first-stage larva, dorsal view.

ABBREVIATIONS: *A* = anterior; *a* = amphid; *al* = albumin; *an* = antenna; *ch* = chorion; *d* = deirid; *es* = ectodermal sac; *gl* = orifice of oesophageal gland; *go* = gonad; *gv* = germinative vesicle; *l* = larva; *la* = lateral ala; *m* = mantle cavity; *p* = papilla; *P* = posterior; *ph* = phasmid; *po* = podocyst; *ro* = respiratory orifice; *s* = shell; *v* = vitelline membrane; *y* = yolk.

The tail was cone-shaped and terminated bluntly. Deirids were not located and lateral alae were absent. The cuticle was thin and devoid of striations.

A molting first-stage larva had the following major dimensions: Length 400 μ. Maximum width 22 μ, near the middle of the body. Oesophagus 131 μ in length. Opening of dorsal oesophageal gland 24 μ from anterior extremity. Oesophageal bulb 18 μ in length and 15 μ in width. Nerve ring 86 μ and excretory pore 96 μ from anterior extremity. Genital primordium 195 μ from anterior end. Tail 66 μ in length.

The Second-stage Larva (*Figs. 6–7, 9–10*)

A typical, early second-stage larva had the following dimensions: Length 480 μ. Maximum width 29 μ, near middle of body. Oesophagus 159 μ in length. Opening of dorsal oesophageal gland 22 μ from anterior extremity. Corpus 82 μ and isthmus 26 μ in length. Oesophageal bulb 29 μ in length and 20 μ in width. Ventrolateral oesophageal glands opening about 99 μ from anterior extremity. Nerve ring 101 μ, excretory pore 124 μ, and genital primordium 338 μ from anterior extremity, the latter consisting of two cells. Tail 98 μ in length. Phasmids 62 μ from caudal extremity.

The anterior end had three large, rounded lips considerably broader than the body of the larva in the head region. The apex of each lip had a pair of papillae and there were at least two other larger, flattened papillae near the base of each lip. The lips contained more tissue than those of the first-stage and were thus much less transparent. The amphids opened well behind the bases of the lips and could not be detected in *en face* preparations. The buccal cavity was similar to that of the first-stage, the apices of the oesophageal lobes having the appearance of minute teeth in *en face* view. The bulb of the oesophagus was more elongate than that of the first-stage larva. The tail was much more attenuated than that of the first stage but the thickness of the most caudal part was variable. Deirids were not located. The cuticle was without alae or obvious striations.

A molting second-stage larva had the following dimensions: Length 614 μ. Maximum width 28 μ near middle of body. Oesophagus 169 μ in length. Opening of dorsal oesophageal gland 26 μ from anterior extremity. Corpus 96 μ and isthmus 21 μ in length. Bulb elongate, 26 μ by 12 μ. Nerve ring 99 μ, excretory pore 108 μ, and genital primordium 341 μ from anterior extremity. Tail 72 μ in length, terminating in three minute projections. Phasmids 45 μ from caudal extremity.

The Third-stage Larva (*Figs. 8, 11–12, 15–17*)

Larval development within the body of the female worm ceases when the third stage is reached and the latter moves into the vagina and passes out the vulva into the mantle cavity. Ten larvae that had emerged into water from a

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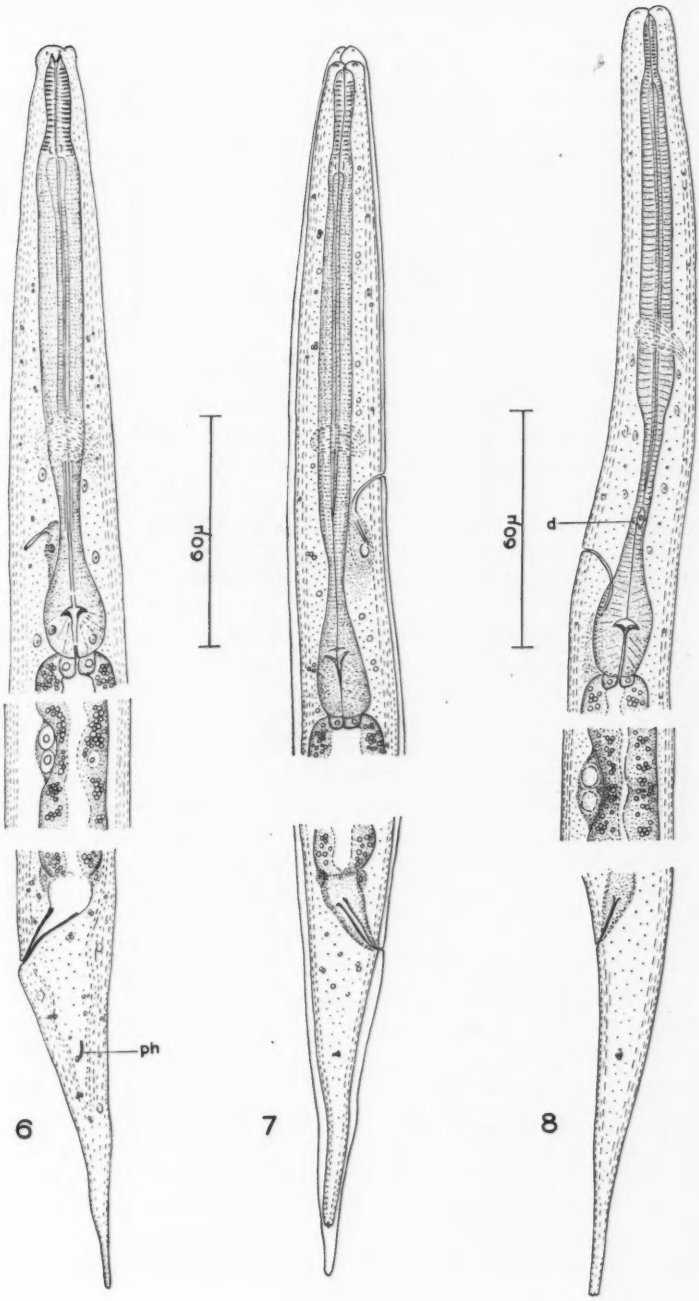
FIGS. 6–8. *Cosmocercoides dukae.*
FIG. 6. Second-stage larva, lateral view.
FIG. 7. Molting second-stage larvae, lateral view.
FIG. 8. Third-stage larva, lateral view.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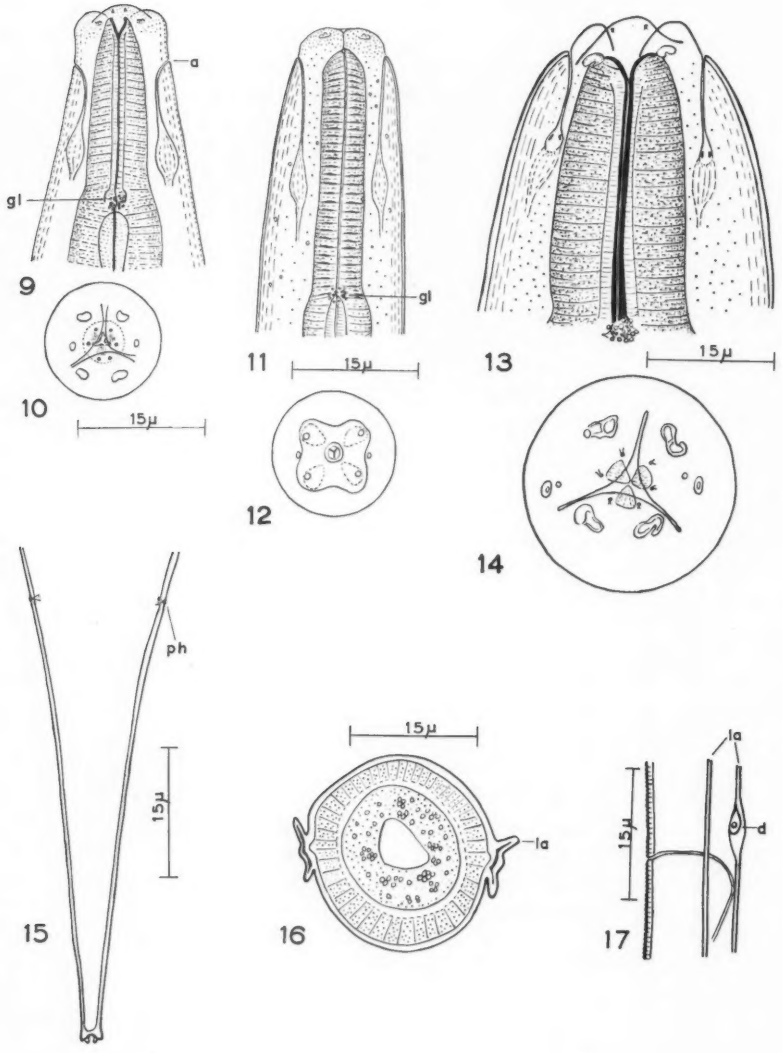


FIGS. 9–17. *Cosmocercoides dukae.*
FIG. 9. Anterior extremity of second-stage larva, dorsal view.
FIG. 10. Anterior extremity of second-stage larva, *en face* view.
FIG. 11. Anterior extremity of third-stage larva, dorsal view.
FIG. 12. Anterior extremity of third-stage larva, *en face* view.
FIG. 13. Anterior extremity of fourth-stage larva, dorsal view.
FIG. 14. Anterior extremity of fourth-stage larva, *en face* view.
FIG. 15. Caudal end third-stage larva, ventral view.
FIG. 16. Cross section through middle of body of third-stage larva.
FIG. 17. Body of third-stage larva in region of excretory pore, lateral view.

female had the following dimensions: Length 752 μ–810 μ. Maximum width 26–28 μ, near middle of body. Oesophagus 186–205 μ in length. Opening of dorsal oesophageal gland 18–20 μ from cephalic extremity. Corpus about 88–90 μ and isthmus 46–48 μ in length. Oesophageal bulb narrow and elongated, about 28–30 μ in length and 14–15 μ in width. Nerve ring 108–117 μ, excretory pore 141–150 μ, deirids 134–144 μ, and genital primordium 412–451 μ from cephalic extremity. Genital primordium about 17–22 μ by 5–9 μ in size apparently consisting of two cells. Tail 86–98 μ in length. Phasmids 49–57 μ from caudal extremity.

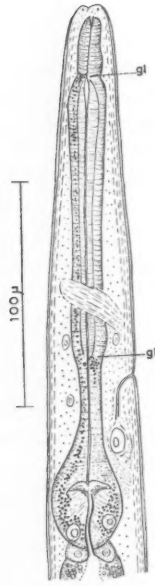
The cephalic end lacked lips but was provided with four large, submedian papillae raised well above the surface of the head. The amphids were well defined and opened about 4 μ behind the anterior extremity. There was no clearly defined buccal capsule and the anterior part of the oesophagus lacked the pointed lobes found in earlier stages. The compactness of the oesophageal tissue obscured the gland cells and the openings of their ducts into the lumen of the oesophagus. The terminal excretory duct was long and clearly defined. The deirids were large and easily seen, especially in dorsoventral view. The tail, terminating in three to four minute projections, was characteristic of the third stage. The phasmids were as clearly defined as the deirids. The cuticle was transversely striated and possessed broad, flanged, lateral alae extending from the region of the opening of the dorsal oesophageal gland to slightly behind the phasmids.

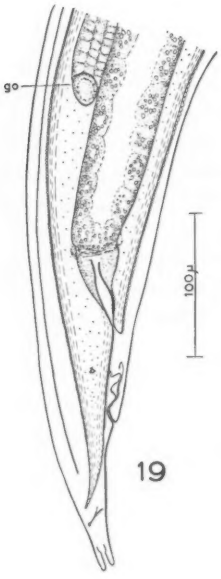
Third-stage larvae lived for weeks in cool water and only developed within the molluscs. At the time of the third molt the sex of the larvae was recognizable but these features will be outlined when the early fourth stage is considered. A molting female third-stage from the mantle cavity of a slug had the following dimensions: Length 950 μ. Maximum width 44 μ. Oesophagus 245 μ in length. Bulb 36 μ in length and 30 μ in width. Opening of dorsal oesophageal gland 24 μ from cephalic extremity. Nerve ring 120 μ, excretory pore 150 μ, and genital primordium 565 μ from cephalic extremity, the latter 123 μ in length. Tail 90 μ in length. Phasmids 58 μ from caudal extremity. The genital primordium was much developed.

The Fourth-stage Larva (*Figs. 13–14, 18–22*)

A typical early fourth-stage larva (female) from the mantle cavity of a slug had the following dimensions: Length 1060 μ. Maximum width 50 μ, near middle of body. Oesophagus 247 μ in length. Opening of dorsal oesophageal gland 28 μ from cephalic extremity. Corpus 129 μ, isthmus 41 μ in length. Bulb approximately 40 μ in length and 32 μ in width. Opening of ventrolateral oesophageal glands 158 μ from cephalic extremity. Nerve ring 130 μ, deirids 160 μ, and excretory pore 167 μ from cephalic extremity. Middle of genital primordium 600 μ from anterior extremity; total length of genital primordium 81 μ. Tail 101 μ in length. Phasmids 54 μ from caudal extremity.

The cephalic extremity had three small lips. A pair of papillae was present on the apex of each lip and four other larger, irregularly shaped, submedian





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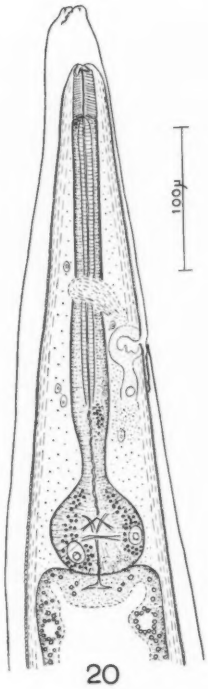

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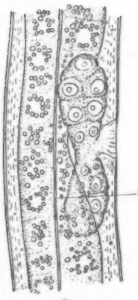

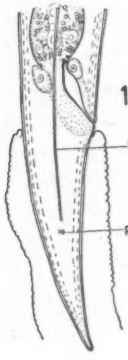

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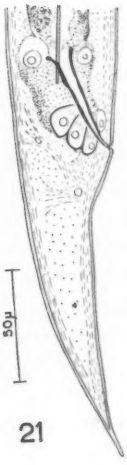

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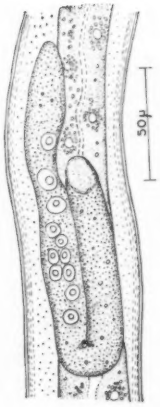

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papillae were present near the bases of the lips (see Fig. 14). A pair of minute papillae lay internal to the amphids; the pores, ducts, and pouches of the latter were easily observed in dorsoventral view, the pores opening at the base of the lips. The oesophageal glands and their orifices were distinctly indicated by the presence of refractory granules. The terminal excretory duct and its cell were prominent. The genital primordium consisted of two multicellular lobes (primordia of the ovaries, oviducts, and uteri) attached to a short, stout neck associated with the body wall (primordium of the vagina); the lobes lay on opposite sides of the intestine. The tail was cone-shaped and terminated bluntly. The cuticle was transversely striated and had rather broad, inconspicuous, simple, lateral alae extending from the region of the orifice of the dorsal oesophageal gland to the phasmids. The deirids were minute and difficult to locate.

The male fourth stage was similar to the female in general dimensions although the tail was more attenuated in appearance. The genital primordium in the male was vermiform in shape and had no obvious attachment to the body wall. In addition, cells which form the spicules and the spicular sheath were present around the rectum and the alae extended only to the anus.

A molting female fourth-stage had the following dimensions: Length 1430 μ. Maximum width 100 μ near middle of body. Oesophagus 353 μ in length. Opening of dorsal oesophageal gland 35 μ from cephalic extremity. Corpus 205 μ, isthmus 51 μ in length. Bulb 62 μ in length and 65 μ in width. Nerve ring 165 μ and excretory pore 198 μ from cephalic extremity. Tail 120 μ in length. Vulva opening 760 μ from cephalic extremity. The gonad extended anteriorly to 200 μ from the junction of the oesophagus and intestine and to within 162 μ of the anus.

Morphology of the Adults of Cosmocercoides dukae Holl, 1928 from Molluscs in Ontario (Figs. 23–33, Table I)

Prolonged study revealed no morphological differences between specimens of *Cosmocercoides* from *D. gracile*, *Z. arborea*, and *D. cronkhitei*. Mature specimens, especially females, are highly variable in size. This variability is somewhat obscured in the following description because, generally, larger specimens were chosen for detailed study. Only measurement of females containing larvae are given below. An indication of the variability in size can be derived from the fact that females containing larvae from *D. gracile* varied from 1.66–4.34 mm in length and 0.15–0.31 mm in width.

FIGS. 18–22. *Cosmocercoides dukae.*

FIG. 18. Early fourth-stage larva (female), lateral view.

FIG. 19. Caudal end molting fourth-stage larva (female), lateral view.

FIG. 20. Anterior extremity molting fourth-stage larva (female), lateral view (same specimen as preceding figure).

FIG. 21. Caudal end early fourth-stage larva (male), lateral view.

FIG. 21*a*. Genital primordium of early fourth-stage male, lateral view (same specimen as preceding).

FIG. 22. Caudal end late fourth-stage larva (male), lateral view.

TABLE I

Major dimensions (in mm) of *Cosmocercoides dukae* from terrestrial molluscs in Ontario

	Host			Range, total	Averages and percentages,* total
	D. gracile	*Z. arborea*	*D. cronkhitei*		
No. specimens	10♂♂ 2♀♀	5♂♂ 4♀♀	5♂♂ 4♀♀		
Length ♂	2.45–2.98	1.85–2.36	1.65–1.92	1.65–2.98	2.3
Length ♀	1.66–4.34	2.56–3.28	2.54–3.65	1.66–4.34	3.0
Width ♂	0.23–0.30	0.16–0.25	0.12–0.20	0.12–0.30	0.22
Width ♀	0.15–0.31	0.18–0.28	0.20–0.30	0.15–0.31	0.24
Oesophagus ♂	0.38–0.46	0.36–0.41	0.35–0.37	0.35–0.38	13–21(17)%
Oesophagus ♀	0.33–0.45	0.42–0.52	0.40–0.50	0.33–0.52	10–19(15)%
Excretory pore ♂	0.29–0.38	0.26–0.29	0.24–0.28	0.24–0.38	10–15(13)%
Excretory pore ♀	0.18–0.35	0.30–0.32	0.26–0.42	0.18–0.42	8–12(10)%
Nerve ring ♂	0.22–0.28	0.16–0.21	0.17–0.22	0.16–0.28	7–12(9)%
Nerve ring ♀	0.14–0.22	0.18–0.26	0.18–0.27	0.14–0.27	5– 8(7)%
Tail ♂	0.13–0.17	0.11–0.13	0.12–0.14	0.11–0.17	4– 8(6)%
Tail ♀	0.14–0.15	0.12–0.17	0.16–0.19	0.12–0.19	4– 8(7)%
Gubernaculum	0.09–0.11	0.07–0.11	0.08–0.10	0.07–0.11	0.09
Spicules	0.22–0.32	0.20–0.28	0.22–0.27	0.20–0.32	0.26
Vulva	0.88–2.42	1.36–1.85	1.39–2.08	0.88–2.42	53–57(55)%
Rosette papillae (R)	12–17	14–17	11–15	12–17	14.6
Rosette papillae (L)	13–18	13–16	13–15	13–18	14.5

*Percentages are ranges and averages of relative positions of fixed points (end of oesophagus, nerve ring, etc.) expressed as percentages of the body length.

General

Anterior end with three prominent lips not clearly set off from body. Internal circle of papillae represented by two minute papillae near apex of each lip. External circle consisting of four large, horseshoe-shaped papillae, two near the base of dorsal lip, and one on base of the ventral side of each ventrolateral lip; in some preparations these papillae seemed double, but in most they seemed single. The ventrolateral papillae of the external circle represented by a rounded, flat papilla, on each ventrolateral lip. Amphids extremely minute, situated immediately behind lips. Oesophagus not clearly divided into procorpus and metacorpus. Part of oesophagus anterior to orifice of dorsal oesophageal gland clearly demarcated from remainder of oesophagus. Oesophagus extended anteriorly into three sharply pointed lobes giving appearance of teeth especially in *en face* view. Cuticle uniformly striated transversely, the striae broken in lateral fields. Flat, rather inconspicuous, lateral alae present extending from anterior third of oesophagus to region of anus in male, and region of phasmids in female. Numerous small, sensory structures scattered over body especially numerous on the dorso- and ventro-lateral surfaces. Caudal extremity pointed.

FIGS. 23–28. *Cosmocercoides dukae.*
FIG. 23. Caudal end mature male, ventral view (from *Z. arborea*).
FIG. 24. Caudal end mature male, ventral view (from *D. gracile*).
FIG. 25. Caudal end mature male, lateral view (from *D. gracile*).
FIG. 26. Caudal end mature female, ventral view (from *D. gracile*).
FIGS. 27–28. Gubernacula, ventral view.

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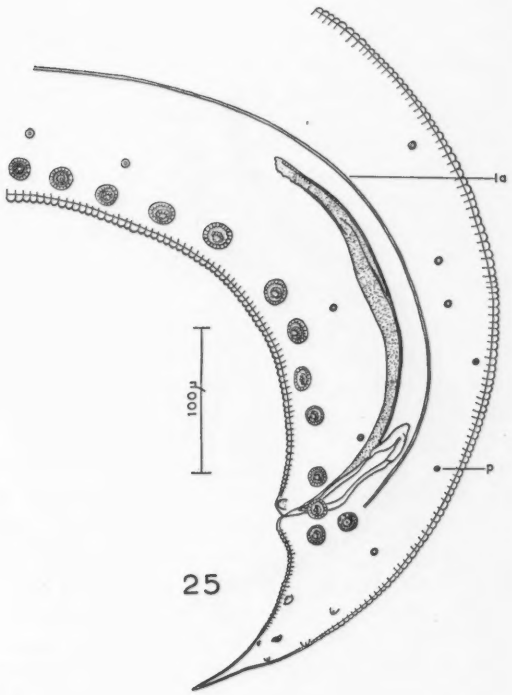

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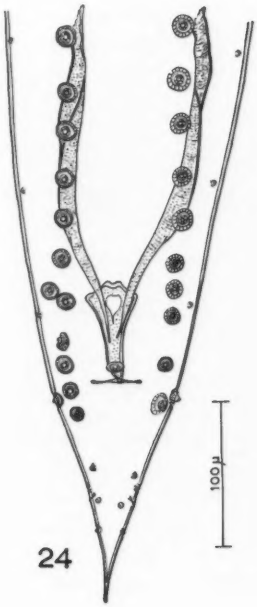

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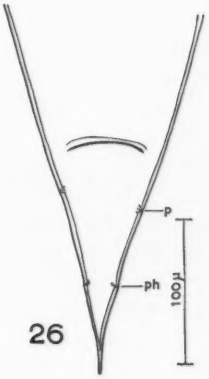

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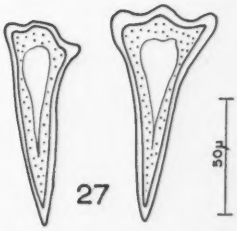

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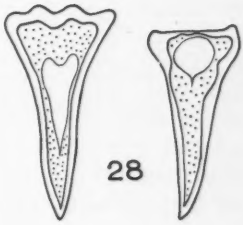

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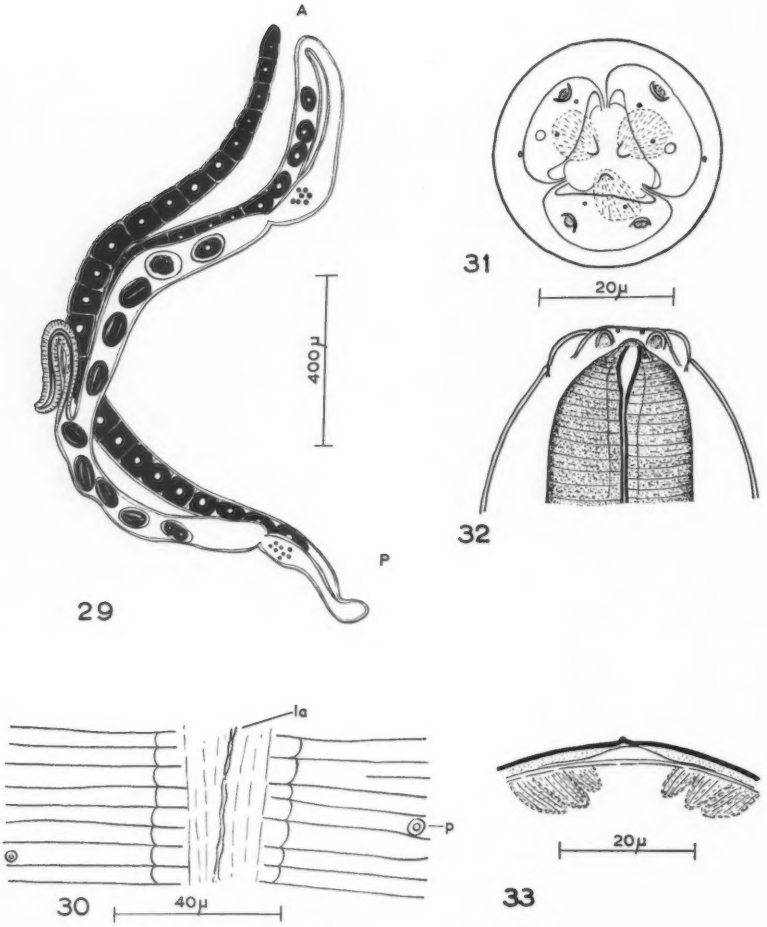


FIGS. 29–33. *Cosmocercoides dukae.*

FIG. 29. Genital tract (semidiagrammatic) of mature female, lateral view (from *Z. arborea*).

FIG. 30. Surface of cuticle near middle of body (male), lateral view (from *Z. arborea*).

FIG. 31. Anterior extremity female, *en face* view (from *Z. arborea*).

FIG. 32. Anterior extremity female, dorsal view (from *D. cronkhitei*).

FIG. 33. Cross section through middle of body of female showing lateral ala.

Male

Opening of dorsal oesophageal gland 35–55 μ from cephalic extremity. Bulb of oesophagus approximately 60–90 μ in diameter. Phasmids 69–74 μ from caudal extremity. Gubernaculum triangular, bluntly pointed, its proximal side flat, bifid, trifid, or quadrifid in form. Spicules slender, delicate, and arcuate; capitulum about one-quarter of total length, although distinction between it and lamina not clear. Number of caudal papillae with rosette formation varying from 11–18 on each side of body; first 3 papillae constantly arranged in a triangular pattern on each side of anus, arrangement of remainder generally linear although some often occur outside the main line. Simple caudal papillae consisting of a single, preanal, median papilla and about 4 pairs of irregularly arranged papillae on distal half of anus. Sperm globular, 10–12 μ in diameter.

Female

Opening of dorsal oesophageal gland 38–51 μ from cephalic extremity. Bulb of oesophagus about 65–100 μ in diameter. Opening of ventrolateral oesophageal glands 220–370 μ from cephalic extremity. Phasmids 52–75 μ from caudal extremity. Vulva a slit at right angles to longitudinal axis of body, sometimes opening on slight swelling. Vagina directed anteriorly for first 200 μ and then posteriorly for a similar distance where it gives off two opposed uteri containing eggs and larvae. Each uterus leads into well-defined spermatheca. Posterior oviduct and ovary directed anteriorly on leaving spermatheca, the ovary terminating in oesophageal region. Anterior oviduct and ovary directed posteriorly for at least two-thirds of their length. Tail pointed, phasmids clearly defined. Pair of ventrolateral papillae about 40 μ behind anus apparently always present.

Hosts

Deroceras gracile Rafinesque, *Zonitoides arborea* (Say), *Discus cronkhitei* (Newcombe).

Locality

Algonquin Park, Ontario, Canada.

Specimens

Nos. 309–310, 322, 331–332, 336–337. Nematode Collection, Ontario Research Foundation, 43, Queen's Park, Toronto 5.

Comments

Holl's (9) original description of *C. dukae* from *Triturus viridescens* is most summary but the few dimensions and features mentioned agree with our data.

Chitwood and Chitwood (6) gave three illustrations of worms referred to as *C. dukae* but they did not mention the host. Their figures of the anterior and caudal ends of a male (Figs. 11C–D) accord with our specimens. Their figure of the head in *en face* view (Fig. 570) departs slightly from our observations, however. Firstly, whereas they show the amphids on the lips, we found them behind the lips. Secondly, the papillae of the external circle (exclusive of the

ventrolaterals) were described as being double, each pair having the form of a figure 8 directed more or less radially to the stoma. Prolonged study of our preparations leads us to think these external papillae are horseshoe-shaped and like those figured by Chabaud and Brygoo (4) for *Aplectana courdurieri*. We are not certain if these papillae can be regarded as double although one or two gave that impression; Chabaud and Brygoo evidently thought each was single in *A. courdurieri*.

Ogren's (11) figure of the anterior extremity in *en face* view shows lips well set-off from the body, amphids behind the lips, and the external circle of papillae (exclusive of the ventrolaterals) consisting of four pairs arranged in a circle about the stoma, the papillae in each pair being well separated. The discrepancies in descriptions of the head may be the result of errors in observation or differences in interpretation since the structures involved are extremely difficult to study.

Ogren (11) claimed lateral alae were absent and the eggs $57 \times 38\ \mu$ in size. Alae are often difficult to see except under oil immersion or in cross section and Ogren may have overlooked them. The dimensions given for the eggs (much smaller than in our material) may be erroneous; the scale on the illustrations of the eggs does not agree with dimensions given in the text. Other features mentioned accord with our specimens and probably his material is conspecific with ours.

Observations on the Transmission of Cosmocercoides dukae

Infective (early third-stage) larvae are almost invariably found with adults in the mantle cavity of molluscs. Six infected *Z. arborea* were placed at room temperature in a Syracuse watch glass covered with an embryological watch glass, and the slime trails left by the moving snails were examined for larvae. Numerous third-stage larvae were found a few hours later in the slime, showing, as already noted by Ogren (11), that these larvae readily leave the mantle cavity.

Third-stage larvae placed near the foot of young snails penetrated into the space between the shell and the foot and were later found in the mantle cavity. On one occasion a young *D. cronkhitei* was placed in a small quantity of water on a depression slide and two third-stage larvae were placed on its foot. In a few minutes the larvae disappeared and the snail was removed from the water and left to dry. This caused the foot to retract. The snail was then replaced in water and a large bubble of air became trapped in the opening of the shell. The respiratory pore of the snail occasionally pulsated and the course of some of the air to the mantle cavity could be observed. One of the larvae was seen making its way up this channel. Three weeks later two advanced third-stage larvae were found in the mantle cavity of this snail.

Fourth-stage larvae were frequently placed near the foot of snails but they showed little inclination to enter the snails although they occasionally became enmeshed in slime. For example, a third-stage and two fourth-stage larvae were placed on the extruded foot of an uninfected *Anguispira alternata* that was reared in the laboratory. The larvae moved easily in the slime on the foot

and disappeared from view, presumably between the foot and the shell. Dissection 2 hours later revealed that only the third-stage larva had reached the mantle cavity; the older stages were not found and may have been eaten. Other attempts to infect snails with stages later than the third gave similar results.

Transmission can also take place through the egg. A third-stage larva was found in 1 of 15 newly hatched *Discus cronkhitei*. The eggs had been removed from the stock culture and from contact with adult snails soon after they had been laid, and there was no possibility of the snails becoming infected after hatching. This mode of transmission was difficult to study in snails, however, because their eggs were opaque and larvae could only be detected after the egg had been destroyed.

The egg of *Deroceras gracile*, unlike those of the snails, is large and transparent (Fig. 34). It is invested in a thick, tough, fibrous outer layer—the chorion. The yolk and the chorion are separated by a thick, hyaline layer of albumin. The yolk, bounded by a tough vitelline membrane, contains the germinative vesicle and an irregular, fiber-like structure of thickened yolk (?).*

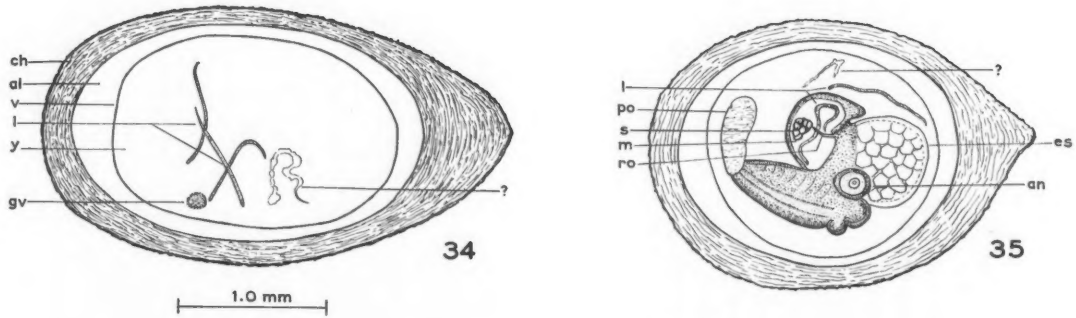
The yolk of 18 of 50 eggs laid by six *Deroceras gracile* in a laboratory culture contained cosmocercid larvae. The germinative vesicle was scarcely developed in 12 eggs and it was possible to count accurately all the larvae present; each egg contained one to three larvae. Six eggs contained advanced embryos which obscured the larvae but one to two were noted in each. Of the 12 little-developed eggs, 2 were fixed and preserved in formalin. The remaining 10 were allowed to complete their development at 16° C and were examined daily to follow the course of development and the position of the larvae. Of these 10, 2 died, presumably as a result of fungus infection which invaded the eggs from the outside. The remaining eight hatched normally but died 11 days later of undetermined causes.†

Larvae entered the mantle cavity of developing slugs when they were as advanced as in Fig. 35. At that time the respiratory orifice and mantle cavity were well formed. The podocyst and ectodermal sac were becoming reduced in size. Larvae may have entered the mantle cavity through the respiratory orifice but this was not observed; embryo slugs at this stage rotated constantly in the yolk and observations were difficult. Larvae may, however, penetrate the tissues since one was noted in the ectodermal sac of an embryo although it was found in the mantle cavity in the fully developed slug.

Dissection of the eight slugs hatched from infected eggs 11 days previously gave some significant results. All larvae were found in the mantle cavity. Two slugs contained early third-stage larvae about 730 μ in length. Four contained fourth-stage larvae varying in size from 925–1100 μ and two contained fifth-stage larvae, one a molting female 1430 μ in length, the other a molting male 1240 μ in length. These results indicate that larvae enter the eggs at different stages. This conclusion was supported by study of larvae from five infected

*The terminology used for slug eggs and developing embryos is from Simpson (13).

†Cause of death may have been the result of overcrowding; slugs require more space than snails of comparable age.



FIGS. 34–35. Eggs of *Deroceras gracile*.
FIG. 34. Newly laid egg containing three larvae of *C. dukae*.
FIG. 35. Embryo within egg, one larva of *C. dukae* still in yolk, the other in mantle cavity of embryo slug.

slug eggs. Two recently laid eggs (germinative vesicle with about eight cells) contained a total of seven advanced third-stage and early fourth-stage larvae ranging in length from 940–1100 μ. A slightly more advanced egg with about 30 cells contained three third-stage larvae 820–900 μ in length, that is, larvae less developed than in the preceding eggs, which were more recently laid. Two advanced, multicellular embryos contained a total of eight third-stage larvae ranging from 740–1045 μ in length. This great variation of larvae in the same egg and in eggs of comparable age shows that more advanced cosmocercid larvae as well as the infective larvae (early third-stage) enter the eggs. The presence of infective larvae shows there was little development between the time the eggs were laid and the hatching of the slug.

The problem of how cosmocercid larvae get into slug eggs was considered. Like Barthélemy (2) we considered the chorion much too tough and thick to be penetrated by larvae. Infective larvae as well as later stages placed on eggs in a moist environment failed to penetrate although they slithered actively over the surface and occasionally probed it. In addition, larvae were not found in uninfected eggs placed for 2 weeks in a culture containing many infected molluscs although the latter were kept close to the eggs. It was thought, therefore, that eggs must get infected in the genital tract of the slug. One adult slug was dissected, and two adult males and four third-stage and five subadult larvae were found in its mantle cavity. A careful dissection of the genital tract was made and one infective larva was found in the albumin gland. One adult slug belatedly laid two eggs on February 8 although the other slugs had ceased laying eggs at least 3 weeks previously. These eggs were heavily infected, one containing 13 larvae, the other 9. The larvae ranged from early third- to fourth-stage. Two mature females and two mature males, along with a number of third-stage larvae, were found in the mantle cavity. No less than 45 larvae were found in the uterus, albumin gland, and ovary. These larvae varied from early infective larvae, about 750 μ in length, to molting third-stage larvae over 1000 μ in length. These results seem to confirm earlier observations of Barthélemy (2), who believed larvae entered eggs being formed in the genital tract.

It has not been determined how larvae reach the genital tract although two possibilities present themselves. Infective larvae could reach the genital tract by penetrating tissues separating the mantle cavity and the tract but larvae have not yet been found in tissues outside the mantle cavity or the genital tract. Larvae may, however, leave the mantle cavity via the respiratory pore, and find their way to the genital opening which they enter. This possibility may be feasible since the genital pore in the slug is especially prominent and located near the head where it would come in contact with larvae in the environment more readily than would the respiratory opening.

Larvae probably grow after reaching the genital tract and Conte and Bonnet's (7) report of mature males and females of *Angiostoma helicis* in the genital tract of *Helix aspersa* may be significant. Larvae of various stages evidently get incorporated into eggs during ovulation, as indicated by the great variability in the size of larvae in newly laid eggs.

Attempts were made to infect young *D. cronkhitei* (varying in age from 2 weeks to 2 months) with infective larvae deposited in water by female *Cosmocercoides* from the mantle cavity of *Deroceras gracile*. Fifteen of these minute snails were removed from cultures (containing only recently hatched snails and eggs) and placed in small drops of water on depression slides. Two to six infective larvae were placed on the foot of each snail and the latter were gradually pushed out of the water. In this way it was possible to keep larvae from floating away from the opening of the snail's shell. The larvae were watched under the dissecting microscope until they had disappeared. There was no way of knowing with certainty if larvae had entered the region between the body of the snail and its shell or only become enmeshed in the thickened slime which formed over the snail's opening after the snail had been pushed out of the water. Snails were finally placed in stender dishes with pieces of moist filter paper and fed lettuce and calcium carbonate. The dishes were kept at 19° C along with dishes containing 15 control snails. The snails were dissected at various times and larvae found in them were studied.

TABLE II

Results of attempts to infect *D. cronkhitei* with infective larvae of *C. dukae* from *D. gracile*

No. snails	No. larvae	Days	Results
1	3	13	One third (860 μ)
2	2	22	One third (900 μ), one fourth (1030 μ)
3	3	23	One fourth (1120 μ)
4	6	23	Two thirds (860 μ)
5	3	25	Three thirds (730–865 μ)
6	3	31	One molting third (1060 μ), one molting fourth (1245 μ)
7–9	3	37	Negative
10	3	39	One fourth (1035 μ)
11–13	3	42	Negative
14	4	52	One molting fourth (1300 μ)
15	4	57	One molting fourth (1370 μ)

The results (Table II) were not as successful as one would have hoped but, considering the difficulty in infecting such young snails experimentally, this was not surprising. Whereas only a single third-stage larva was found in 1 of the 15 controls, larvae were found in 9 of the 15 experimental snails. Development was evidently variable. An early fourth-stage larva was found in a snail 22 days after the snail was infected but it also contained a third-stage only 900 μ in length. Similarly, two snails dissected 23 and 25 days later contained only third-stages, most of which, however, were well past the infective stage in development. The final molt was noted in a larva from a snail dissected 31 days after it was infected, but this snail also contained a larva in the third molt. Few larvae were recovered from the snails and the results do not show clearly either the rate of development or the times of the molts but they do suggest that the *Cosmocercoides* in slugs can be transmitted to snails.

Relationship of Cosmocercoides in Amphibians to Those in Molluscs in Ontario

During August and September of 1959 numerous amphibians were collected in Algonquin Park and the Caledon Hills; most were taken from the former locality. Many were autopsied soon after capture but a number were kept at low temperatures in the laboratory and examined weeks or months later for the presence of *Cosmocercoides*. Species infected were as follows:

Rana pipiens (6 of 32 examined), *R. clamitans* (7/25), *R. catesbeiana* (1/3), *R. septentrionalis* (1/7), *Bufo americanus* (2/5), *Ambystoma jeffersonianum* (1/1). Only one to six specimens were found in each infected amphibian and females with mature eggs or free larvae were rare. All specimens were found in the rectum.

Careful morphological study revealed no differences between the nematodes in amphibians and molluscs. It was noted, moreover, that several frogs dissected a day or two after capture contained remains of such terrestrial molluscs as *D. cronkhitei*, *Z. arborea*, and *Deroceras* sp., raising the possibility that frogs acquire *Cosmocercoides* by eating molluscs. This possibility was strengthened by the fact that frogs kept in the laboratory for long periods tended to lose their infections even though kept at such low temperatures that defaecation was infrequent. Thus 32% of 41 amphibians were infected when autopsied before November 1 but only 23% of 32 examined thereafter were infected. Since adult and larval *Cosmocercoides* are so hardy they can survive for weeks in tap water outside the mantle cavity, it was suspected they might also survive passage through the gut of amphibians. A few experiments were conducted to test this possibility.

(i) Four *Z. arborea* which, as a result of dissections of snails from the same lot, were assumed to be infected with *Cosmocercoides*, were forced down the throat of a young *R. pipiens* collected a week previously in Algonquin Park. At autopsy 24 hours later, three snails were found in the stomach; the shells were partially digested but the bodies were still intact. No nematodes were found in the snails, or the stomach and intestine of the frog but four mature females, four males, and a number of larvae were found in the rectum. These worms were active and lived for several days in tap water. The number of worms present was greater than found in other frogs, suggesting that at least some of the nematodes had come from the ingested snails.

(ii) Seven days after capture in Algonquin Park, four young *R. pipiens* were each given four *Z. arborea*, some or all of which were assumed to be infected with *Cosmocercoides*. Each frog was placed in a small jar containing a quarter inch of tap water. The experimental frogs, along with four controls, were kept at 15° C and the water in each container was examined daily for the presence of nematodes.

Over a period of 2 weeks a total of 5 mature males, 12 mature females, and 5 third-stage larvae were found in water containing the experimental frogs. All the worms were active and a number was kept for several days in cool water; some females deposited eggs and (or) larvae. Only two mature and four immature female cosmocercids were found in the water containing the controls.

No cosmocercids were found in any of the frogs autopsied 2 weeks after the start of the experiment.

(iii) Snails fed to frogs in the preceding experiments contained infective larvae as well as adults. However, two attempts were made to infect frogs with infective larvae only. On October 9, 50 third-stage larvae deposited by female *Cosmocercoides* from *Z. arborea* and *D. cronkhitei* were pipetted into the oesophagus of a young *R. pipiens* collected on September 22 in Algonquin Park. The frog was kept at 15° C in a jar containing a small quantity of water, which was examined daily for the presence of nematodes. Fourteen days after larvae were given to the frog, a single, active third-stage larva was found in the water. Fourteen days later, seven active third-stages were found. No cosmocercids were found in the frog autopsied 46 days after it was given larvae.

A similar attempt was made to infect another young *R. pipiens* using 65 third-stage larvae. Only one active third-stage larva was found (11 days later) in water surrounding the frog and no cosmocercids were found at autopsy 43 days after the start of the experiment.

(iv) Finally, a young *R. pipiens*, collected in the Caledon Hills and kept without feeding at low temperatures in the laboratory for 6 months, was given nine large *D. cronkhitei* and *Z. arborea*, all, or most, of which were heavily infected with *C. dukae*. The frog was kept at room temperature (22–23° C) in a small quantity of water. Cosmocercids were first passed on the day following feeding. On the second day, four partially digested snails were defaecated. Some of the cosmocercids were dead but most of these were found in the mantle cavity of one of the partially digested snails. Worms, along with fragments of snail shells, continued to be passed up to the 13th day after the snails had been fed to the frog. Over this period the following worms were passed: (*a*) *living*—81 third-stages, 4 fourth-stages, 9 males, 18 females; (*b*) *dead*—2 fourth-stages, 6 males, 12 females. Some eggs and some first- and second-stage larvae found in faeces were assumed to have been released during digestion of some female worms. This frog was autopsied 30 days after snails were fed to it, 7 days after the last worms enumerated above were passed. One female without eggs or larvae and three fourth-stage larvae were found in its rectum.

The results suggest that *Cosmocercoides* from molluscs in Ontario do not establish themselves in *R. pipiens* but pass through the gut and, at intervals, out in the faeces. Presumably specimens passed by controls in the second experiment mentioned above were from molluscs eaten a short time before the frogs were collected. The presence of dead worms, especially in partially digested snails passed by frogs, shows that *C. dukae* is not refractory to digestion in the frog's gut. The results in experiment (i) indicate, however, that usually cosmocercids rapidly leave the mantle cavity of ingested snails and make their way to the frog's rectum where they can live indefinitely unless passed in the faeces. Those worms that fail to leave the digesting snail are killed *in situ*, and remains of them can ultimately be found in the frog's faeces.

Harwood's (8) account of the morphology of *Cosmocercoides variabilis* in amphibians and reptiles in Texas agrees with the species found in Ontario. The incidence of infection was, however, much greater than found in Ontario. For example, 86.5% of *Bufo valliceps*, 83.3% of *Storeria dekayi*,* and 4–67% of the remaining eight species examined were infected. If these nematodes had been acquired from eating molluscs it would mean the latter are extremely important items in the diet of amphibians and certain reptiles in Texas at least during the period (unfortunately not specified) when Harwood collected them.

According to Harwood, eggs containing larvae were deposited by the female worm and found in fresh faeces of the host. The eggs hatched in a few hours and the first-stage larva (350 μ in length) developed in cultures of the host's faeces. The first-stage was said to have a sharply pointed tail but this is perhaps erroneous. He overlooked the first molt and his so-called first stage may have been the second stage. His description of the third stage accords with our studies except that he stated that three lips were present. It is difficult to know how much importance to attach to Harwood's observation that female *variabilis* laid eggs rather than infective larvae. We have already noted that female worms removed from molluscs habitually discharged part of the contents of their uteri whether or not the latter contained poorly segmented eggs, eggs with larvae, or free larvae. Eggs without larvae failed to develop but those with first-stage larvae generally hatched and the latter developed to the infective stage in tap water. A female, the uteri of which were packed with eggs, was once taken from the rectum of a young *R. pipiens* that had been fed four *Z. arborea*. When placed in tap water this worm discharged numerous eggs, many of which contained first-stage larvae that subsequently ruptured the egg capsule and developed to the infective stage. The female was kept under observation and 2 weeks later her uteri contained third-stage larvae, 17 of which were subsequently deposited. We have not observed eggs or first- or second-stage larvae in the mantle cavity of molluscs although Ogren (12) reported rhabditiform larvae in the mantle cavity of *Deroceras gracile*. Our observations lead to the conclusion that development to the infective stage in molluscs usually takes place in the uteri of the female worm but, when she is disturbed (i.e. by transfer from the mantle cavity to water), eggs and early larval stages, if present, may be discharged. Harwood's observations may perhaps be explained in this way.

Harwood's attempts to infect 10 *Bufo valliceps* were, as he mentioned, inconclusive. One toad was uninfected 11 days after 200 larvae had been placed on its belly. Six toads were injected subcutaneously with 50–300 infective larvae. The lungs of three had one to three immature worms when examined 2–6 days later. Three immature worms were found in the rectum of one 30 days later and 4 immature worms in the lungs and 11 in the rectum of another examined 11 days later; no nematodes were found in one inoculated toad. Attempts to infect toads by the oral route were no more successful. Three toads were each given 50–300 larvae orally. Two were negative 3–5 days later and a single larva was found in the lung of one examined 6 days later.

*Which, according to Logier (10), has a great fondness for slugs.

Unfortunately, Harwood did not give the history of the toads used in his uncontrolled experiments and the possibility that they were caught in the field and already infected cannot be ruled out. Moreover, nowhere does he figure or give dimensions of larvae found in the lungs, and one wonders if he may not have found immature *Rhabdias*, which are common in toads and can be confused with *Cosmocercoides* if not examined carefully under the microscope. In any event we do not think his results can be taken as proof of a lung migration. We think the results of Harwood's attempts to infect toads with *Cosmocercoides variabilis* may accord rather well with the notion that his species is basically a parasite of molluscs figuring in the diet of amphibians and reptiles, but work in Texas will be needed to settle this question.

Discussion

Chabaud and Brygoo (3, 4) have called attention to the zoological importance of the Cosmocercidae, believing them primitive forms linking free-living rhabditids to ascarids and oxyurids. The fact that *C. dukae* has not yet developed the consistent and more or less fixed pattern of transmission characteristic of most nematode parasites suggests it is primitive. A further unusual feature of this species is the development of larvae to the infective stage in the uterus of the female worm. One would expect superinfections in molluscs would be inevitable since infective forms are continually being deposited in the definitive site. Yet, heavy infections have not been found in molluscs in Ontario. A balance is probably maintained by larvae continually leaving the mantle cavity and making their way to the external environment. Usually, it is the third-stages which leave, but observations of Ogren (11) indicate later stages may leave as well. Another outstanding feature of *C. dukae* is the ability of all stages to survive indefinitely in the external environment and to pass unharmed through the gut of amphibians. The latter must be important factors in the dissemination of *C. dukae*. Mature females reaching the external environment may lay eggs containing first-stage larvae which develop to the infective stage, or development to the latter stage can take place in the uterus and larvae can be deposited by the female or break out of the carcass if the female dies. Finally larvae can infect the genital tract of molluscs, be incorporated into the eggs, and penetrate into the mantle cavity of the embryos. Thus, considering the various ways this species can be disseminated and transmitted, it is scarcely surprising to find it so abundant in natural populations of molluscs.

Acknowledgments

We express our appreciation to Dr. C. D. Fowle and Mr. R. O. Standfield of the Department of Lands and Forests for facilities at the Wildlife Research Station in Algonquin Park. We are also particularly grateful to Prof. F. P. Ide, University of Toronto, Dr. G. F. Bennett, Ontario Research Foundation, and Mr. M. Wood, McMaster University, for kindly assisting in the identifications of molluscs and amphibians. Dr. Bennett collected many of the amphibians used in this study.

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BARNACLE SHELL FIGURES AND REPAIRS[1]

IRA E. CORNWALL[2]

Abstract

More interlaminate and ala figures are shown. Figures of subspecies were examined to find variations that would distinguish them. The structure of the shell was examined by decalcification. It was found that the pores in the shell of *Balanus nigrescens* are nearly filled with tissue and not shelly matter. Other species examined have only a thin layer of tissue lining the parietal pores. Tests were made that show that the structure of the shell is crystalline and not amorphous.

Material

Material for this paper was obtained from the following: The Australian Museum, Sidney, Australia; U.S. National Museum, Washington, D.C.; Dr. L. B. Holthuis, Rijkamuseum van Natuurijke Historie, Leiden, Holland; Dr. G. C. Carl, Director of the B.C. Provincial Museum, Victoria, B.C.; Dr. Dora Priaulx Henry of the Department of Oceanography, University of Washington, Seattle, Washington; Mr. A. C. Frue, Miami Beach, Florida; Dr. C. H. Edmondson, Director of the Bernice P. Bishop Museum, Honolulu, Hawaii.

The material used for decalcifying the barnacle shells is the Analar reagent commonly known as EDTA.

Discussion

The figures in the shells of sessile barnacles, the interlaminate and the ala figures, are undoubtedly very stable in any one species. Subspecies may differ from species in very minor anatomical or ecological features, but the interlaminate and ala figures remain unchanged. The writer recently examined many authentic subspecies of *Balanus amphitrite* Darwin to try and find some variation in the figures that could be used to distinguish them, but in all those examined there was only the usual variation due to distortion; there was no greater variation between the subspecies than is usually found in different parts of any one shell. In every specimen examined the figures were of the same type as the figures found in the species from which the subspecies was derived. It seems probable that some difference might be found if a large number of specimens were examined, but only a few specimens of each subspecies were available.

In two previous papers the figures found in fossil remains of several species were shown and in every case the figures were the same as those of the recent specimens of that species.

This paper is really a continuation of my previous papers on the figures found in the shells of sessile barnacles. It is strange that after Alessandri (1)

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found these figures that so little use was made of them. I have found, and proved, that they are true indicators of species and have shown numerous figures that can be used to identify fossil and recent sessile barnacle shells. Of course there are many more figures of both recent and fossil barnacles that should be shown. The distinctness with which the figures can be seen varies greatly in the different species. There are some in which a slight trace of color makes the figure stand out clearly; then in others there is a definite difference in the structure of the shell that causes the light to be reflected at a different angle from the portion of the shell forming the figure. The most difficult ones to see are those that have very little difference between the portion of the shell forming the figure and the rest of the shell.

In making the sections of barnacle shells to see the figure it has been frequently noticed that a slight variation, or change, in the angle at which the light strikes the section makes the figure clearer or makes it almost disappear. A remarkable instance of this was seen when making a section of *Balanus decorus* (4, Fig. 1). When the section was first placed on the microscope table no figure could be seen, but as the table was turned the figure gradually appeared. This seems to show that the structure of the shell is crystalline. If the shell is formed of minute crystals it seems likely that the crystals forming the body of the shell may be arranged at a certain angle and those that are in the portion of the shell forming the figure may be at a slightly different angle. Then the result would be that when the light was reflected from both areas of the shell at the same angle the figure could not be seen. But when the angle of the light from the two areas was different the figures could be seen. This effect would not be apparent if the shell was amorphous.[3]

When I first found a figure in the barnacle shell, I put it away for some time and it got dry. When later I looked at the section the figure was gone. I found that by wetting the section the figure would appear again. Benzyl alcohol is remarkably effective with some figures, but not with all.

Darwin (5, p. 56) when working on shell growth wrote that he boiled the shell in acid. This, of course, would destroy both the shell and the tissue that was in it. Decalcification would seem to be a better method as it would show the tissue that was in the substance of the shell and really forms part of it, and also the tissue that supplies the nourishment for the growth of the shell. There is a good method for doing this that has been in use for some time. It removes the shelly matter, calcite, and leaves the tissue as it was before the removal of the calcite. This is the complex reagent EDTA which was mentioned previously (in the section Material). The great difficulty with this method was that the tissue was left unsupported after the calcite was

[3]Since writing the above I have received conclusive proof that the structure of the barnacle shell is crystalline. Dr. V. J. Okulitch, of the University of British Columbia, kindly offered to have X-ray photographs made of barnacle shells. I sent him specimens of *Balanus nubilus* and *B. crenatus* and Dr. R. M. Thompson, of the Department of Geology, made X-ray powder diffraction photographs of the two specimens and wrote to me as follows: "The ultimate test for crystallinity is an x-ray photograph. Crystalline substances give a pattern but amorphous substances give nothing." Also "Both specimens are crystalline and both give the x-ray powder pattern of calcite with no extra lines."

removed and the slightest motion would disturb it. A method was finally developed to overcome this difficulty. A shelf was made that was just high enough for the table of the microscope to pass under it. On this shelf was fastened the glass dish in which the decalcifying was done. The decalcifying solution was removed drop by drop with a medicine dropper and the new solution added in the same way. By using this arrangement the process of decalcifying could be watched through the microscope at any time without disturbing the object being worked on.

Obviously the growth of the wall plates and base takes place at their juncture. To see as much as possible of this process a piece of shell was cut from a large shell of *Balanus nubilus* showing the juncture of a wall plate with the base. When this piece was cut the edge of a layer of tissue could be seen near the base of the wall plate and just above its juncture with the base. This piece of shell was then decalcified and what appeared to be a layer of tissue proved to be made up of three layers. These three layers were close together. The center layer was light-green and looked as if it had been alive. The layers of tissue on each side of the green layer were very thin and colorless and they appeared to have been cast off from the center layer at each growth period as portions were distributed all through the substance of the shell.

Another experiment was tried to see how the thin colorless tissue was distributed in the substance of the shell. A section of the wall plate was cut at right-angles to the line of growth in the same way as it is cut to show the interlaminate figure. This section was placed in the decalcifying fluid (EDTA) for 2 hours; this removed a thin layer of calcite and exposed the edge of the colorless tissue that was distributed all through the shell. This was then stained with methylene blue. This tissue appeared to have been much shrunken when it was absorbed into the growing shell for it did not appear as a line but as a series of irregular dots.

A number of specimens of *Balanus nigrescens* Lamarck were sent to me from the Australian Museum in Sidney, Australia. One has a hole bored through one of the wall plates. This hole is about one-eighth inch in diameter and looks like the hole bored by a polinices. Apparently the barnacle was not killed as the hole had been repaired. The repair was made in the way that the wall plates are built. A layer of columnar shell was arched over the inside of the hole, then a layer of solid shell was built over it forming a smooth round lump, or projection, inside the shell. This is shown diagrammatically in Fig. 11. There are a number of small protuberances inside the shell, showing that it had been bored and repaired several times.

The interlaminate figure of *Balanus nigrescens* (3, Figs. 23–24) and the ala figure (Fig. 1) are much alike. This is the only species, so far examined, that has both these figures of the same type. Another peculiar thing about this species is that its parietal pores are filled with tissue. Darwin states in his monograph (5, p. 211) "The tubes are often thickly lined or almost filled up solidly with blue shell." I decalcified a wall plate of *B. nigrescens* by the method mentioned before and found that the lining of the tubes is tissue and

not shelly matter. After many tries I succeeded in getting the tissue to remain in position after the removal of the calcite (Fig. 16). The small pieces set at right angles to the filling of the parietal tubes are the pieces of tissue that were in the tubes in the ala. Figure 17 is an enlarged cross section of the filling from the parietal tubes. Figure 18 is an enlarged cross section of the lining of the tubes in the ala. They are not filled but are lined with two thin layers of tissue.

The ala figure of *B. eburneus* Gould is like the ala figure of several other species, but it has one distinguishing mark, one end has two little ear-like projections. The specimen examined was from Wood's Hole, Mass., U.S. National Museum specimen No. 10753.

Several specimens of *Balanus hesperius* Pilsbry (3, Figs. 19–20) were collected at Swanson Channel by Dr. G. C. Carl, Director of the B.C. Provincial Museum at Victoria, B.C. They have the usual tear-drop figure of that species (3, Fig. 19). The ala figures are simply a row of irregular dots (Fig. 2). They are arranged singly in some places and in pairs in others.

The ala figures of *B. perforatus* shown in Fig. 5 are rarely found. Those shown in Fig. 4 are typical of the species, but the white spot that is always seen with the interlaminate figures seems to be a sure indicator of *B. perforatus* as it has not yet been found in any other species.

The ala figure of *B. laevis nitidus* (Fig. 6) is much like that of *B. crenatus* but it is wider and the ends are more nearly square. The edge of the *B. crenatus* figures are scalloped and those of *B. l. nitidus* are nearly straight. The specimen examined was U.S. National Museum No. 9224, from Peru.

When I first examined the section of *B. laevis nitidus* to see the interlaminate figures, nothing could be seen but the round dots; however, when I applied benzyl alcohol the stem and its connections with the dots could be clearly seen. Benzyl alcohol does not always have this effect, but I always try it when a figure is faint.

The ala figure of *Balanus balanus* (Linnaeus) (Fig. 7) is much like that of *B. engbergi* (4, Fig. 24) but the figures are much more irregular. The specimen examined was from The Netherlands.

B. balanus is one of the best known barnacles and has been given many names by different authors. Pilsbry (7, p. 149) lists nine names that it has been given. Some still follow Darwin (5, p. 256) and call this species *B. porcatus*. But it must be remembered that his monograph was written over 100 years ago. At the present time, as Pilsbry states in his monograph (7, p. 151), "The specific name is now held to be the permanent part of the binominal combination." Linnaeus described it as *Lepas balanus* in 1758 and da Costa described it as *Balanus porcatus* 1778. According to the modern rules of nomenclature the correct name is *Balanus balanus* (Linnaeus) with Linnaeus's name in parentheses to show that the generic name has been changed. Linnaeus's original specimen is preserved in the collection of the Linnean Society of London and it is shown in Pilsbry's monograph (7, Plate 33).

Balanus glandula Darwin (Fig. 8) has a row of figures in the ala that are of various shapes and they are set at different angles. The parietal pores of this species are small and completely filled with white powder. The specimen figured is No. 87669 from the U.S. National Museum. It was from San Juan Island, Washington.

Balanus tintinnabulum californicus Pilsbry (Fig. 9) was collected from the intertidal zone at Pacific Grove, California. The ala figure resembles that of *B. amphitrite* (3, Fig. 11) as the projections, or arms, of the figure are all on one side of the stem. These projections are sharp-pointed and vary in number. The specimen was loaned by the B.C. Provincial Museum, Victoria, B.C.

There is considerable variation in the size of the parietal pores of *B. improvisus* and also in the size of the figures. The ala figure is rarely found. I have only found two of them, one from a specimen from the Esquimalt Lagoon, Vancouver Island, B.C., and the other from U.S. National Museum specimen No. 51788. This is shown in Fig. 10 and was from Hecate Strait, B.C.

The interlaminate figure of *Balanus amaryllis* Darwin (Fig. 12) is much the same type as that of *B. eburneus* (3, Figs. 43–45) and several other species in which there is a main stem and branches. The stem and branches in the figure of *B. amaryllis* are thicker than any so far examined and there is an uneven number of branches on each side of the stem. Some of the branches are opposite and some alternate. There is another peculiarity about the figures of this species: they are set in the shell at various angles. The specimen examined, and figured, was collected by Mr. H. E. Wyeth of the cable ship *Restorer* from 100 fathoms off Sorogon Bay, Philippines, and was loaned by the B.C. Provincial Museum, Victoria, B.C.

Some time ago I received a number of barnacles from the U.S. National Museum for examination (lot 89298). They were mostly *B. psittacus* (3, Fig. 31) and were from Antafagasta, Chile. Among them was one empty shell that looked different from the others. It had the interlaminate figure (Figs. 13–14) that is always found in the shell of *B. decorus* (2, Figs. 1–2). But the outline of the parietal pores is so different from that in any *B. decorus* so far examined that the shell may prove to be of a variety or subspecies. But this cannot be determined till a whole specimen is examined.

The interlaminate figure of *Elminius plicatus* Gray (Fig. 15) is formed of a row of irregular ovals. The specimen examined was sent to me by Dr. C. H. Edmondson, Director of the Bernice P. Bishop Museum in Honolulu, and was collected near Wellington, New Zealand. The interlaminate figure shown for this species by Gruvel (6, p. 365) is quite different from the figure in this specimen.

The interlaminate figure of *Balanus aquila* Pilsbry (Fig. 19) is much the same type as the figure of *B. regalis* (4, Figs. 12–13) but the branches are placed at a different angle and there are no fine light lines between some of the branches as in *B. regalis*. The specimen figured was sent to me by the U.S. National Museum, specimen No. 87936. It was from San Antonia Del Mar, Lower California.

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EXPLANATION OF FIGURES

PLATE I

FIG. 1. Ala figures of *Balanus nigrescens* Lamarck. From Wilson's Promontory, Victoria, Australia. Collected by Miss E. Pope of the Australian Museum, Sidney, Australia. Identified by I. E. Cornwall.

FIG. 2. Ala figure of *B. hesperius* Pilsbry. Collected at Swanson Narrows, B.C., by Dr. G. C. Carl, Director of the B.C. Provincial Museum, Victoria, B.C. Identified by I. E. Cornwall.

FIG. 3. Ala figure of *B. eburneus* Gould. U.S. National Museum specimen No. 10753, from Wood's Hole, Mass. Identified by H. A. Pilsbry.

FIG. 4. Ala figures of *B. perforatus* Bruguiere. From Bay of Naples. Identified by Dr. L. B. Holthuis.

FIG. 5. These are the ala figures from another part of the same ala as those shown in Fig. 4.

FIG. 6. Ala figures of *B. laevis nitidus* Darwin. U.S. National Museum specimen No. 9224, from Peru. Identified by H. A. Pilsbry.

FIG. 7. The ala figures of *B. balanus* (Linnaeus) from coast of Netherlands. Identified by Dr. L. B. Holthuis.

FIG. 8. Ala figure of *B. glandula* Darwin. U. S. National Museum specimen No. 87669, from San Juan Island, Wash. Identified by Dr. Dora Priaulx Henry.

FIG. 9. Ala figure of *B. tintinnabulum californicus* Pilsbry. From Pacific Grove, California. Identified by I. E. Cornwall.

FIG. 10. Ala figure of *B. improvisus* Darwin. U.S. National Museum specimen No. 51788, from Hecate Strait, B.C. Identified by H. A. Pilsbry.

FIG. 11. Diagram of repair hole in shell of *B. nigrescens*.

PLATE II

FIG. 12. The interlaminate figures of *B. amaryllis* Darwin. Collected by Mr. H. E. Wyeth of cable ship *Restorer*, from 100 fathoms, off Sorogon Bay, Philippines, and loaned by the Provincial Museum, Victoria, B.C. Identified by I. E. Cornwall.

FIG. 13. Interlaminate figures of *B. decorus*. U.S. National Museum. One shell with lot 89298 *B. psittacus*. Identified by I. E. Cornwall.

FIG. 14. Another part of the shell shown in Fig. 13.

FIG. 15. The interlaminate figure of *Elminius plicatus* Gray. From near Wellington, New Zealand. Identified by Dr. Dora Priaulx Henry.

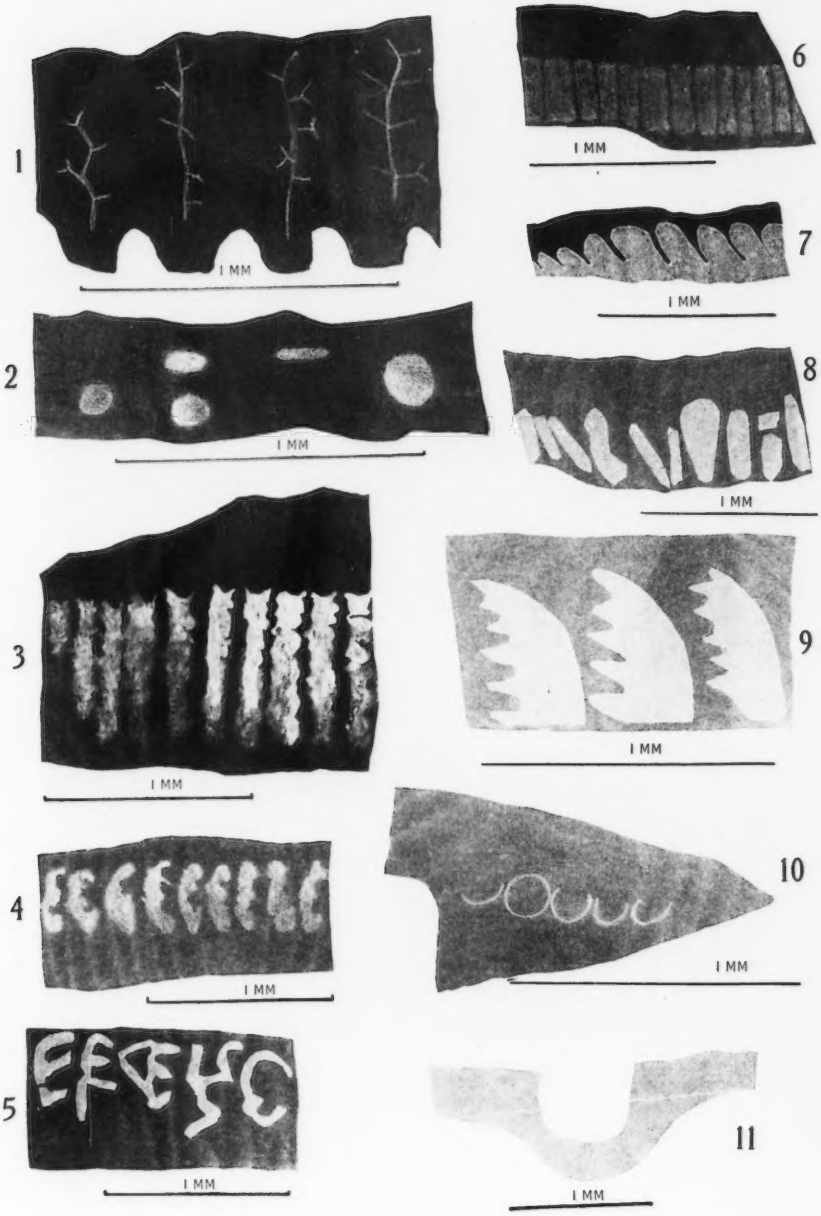
FIG. 16. The tissue that filled the parietal pores in the same position that it was in before the removal of the calcite. The small pieces were in the pores of the ala.

FIG. 17. A cross section, enlarged, of the tissue that filled the parietal pores.

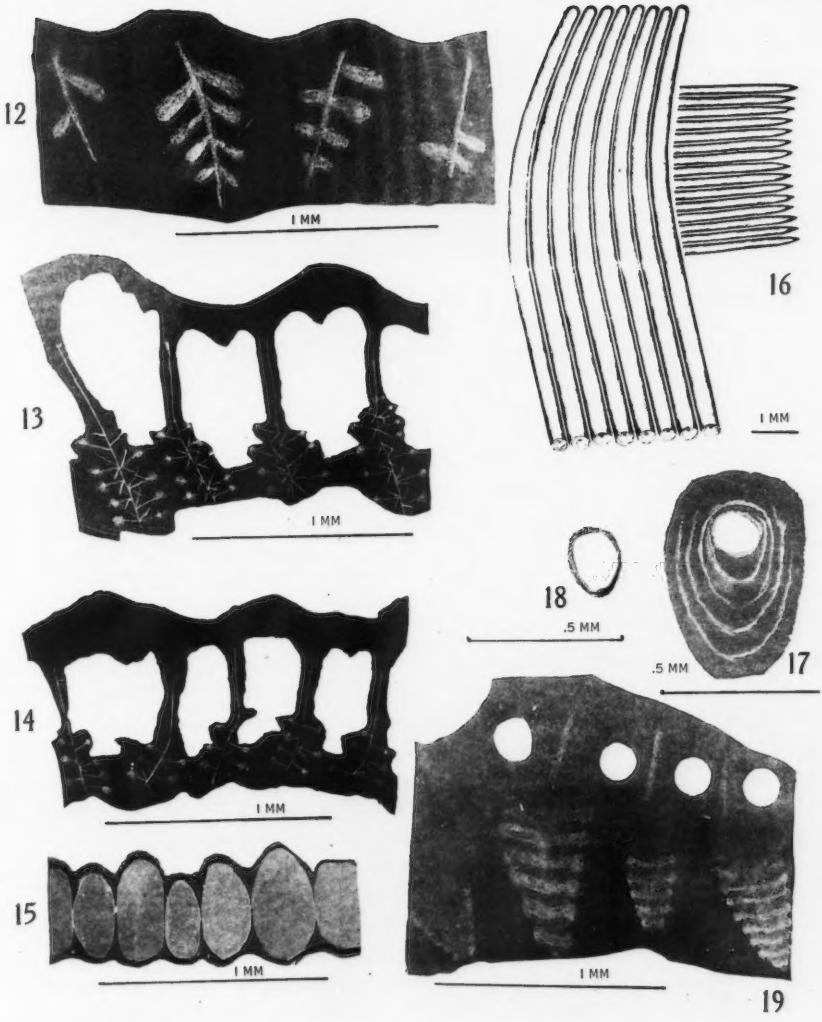
FIG. 18. A cross section, enlarged, of the tissue that filled the pores in the ala.

FIG. 19. The interlaminate figures in the shell of *B. aquila* Pilsbry. U.S. National Museum specimen No. 87936, from San Antonia Del Mar, Lower California. Identified by Dr. Dora Priaulx Henry.

NOTE: Figs. 1–19 follow.



Cornwall—Can. J. Zool.



Cornwall—Can. J. Zool.

HELMINTH PARASITES FROM VERTEBRATES TAKEN IN THE SUDAN (EAST AFRICA)[1]

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Abstract

This paper comprises a list of the parasites from vertebrates collected in the Sudan (East Africa) by the U. S. Naval Medical Research Unit No. 3.

The helminths upon which this study is based were obtained from a total of 90 hosts examined in the Sudan. This is one of several extensive collections taken by the U.S. Navy in biological and geomedical studies in Africa and the Middle East. The pentastomid parasites from the collections have been described by Self and Kuntz (1) and the different categories of ectoparasites have been handled by Dr. Harry Hoogstraal and associates of the U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Materials and Methods

Vertebrates examined in this study were taken in the Tonj District of Bahr El Ghazal Province. Reptiles were collected by the junior author, while the larger mammals and birds were obtained with the help of members of the Sudan Veterinary Service responsible for the control of various hosts as a part of a tsetse fly eradication programme.

Most of the smaller hosts were taken alive, then killed shortly before they were examined for parasites. Larger mammals and birds were examined 1 to 3 hours after they had been shot. The visceral systems were examined separately. After a macroscopic examination the organs were cut into smaller parts. Tissues were macerated with scissors and small forceps, then were washed in several changes of fresh water by being shaken in capped fruit jars. Frequently some of the smaller helminths were recovered from the sediment of washings.

All helminths were killed by immersion in hot water, then transferred to containers with FAA (formalin – acetic acid alcohol) fixative. After 8 to 15 hours specimens were transferred to bottles or vials with 70% alcohol plus 2% glycerine.

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Helminths were forwarded to the Institute of Parasitology at Macdonald College for taxonomic studies. Representative specimens are being deposited in the collections of the Institute of Parasitology and in the Helminthological Collections of the U. S. National Museum at Beltsville, Maryland.

In the following list, the letters N, C, or T before the name of a parasite signify nematode, cestode, or trematode, respectively.

Parasites

In MAMMALS

(a) Insectivores

Atelerix pruneri oweni

C *Mathevotaenia symmetrica* (Baylis, 1927) Akhumian, 1946
N *Physaloptera dispar* von Linstow, 1904
N *Spirura* sp. (female only)

(b) Rodents

Arvicanthis niloticus

C *Inermicapsifer arvicanthidis* Kofend, 1917

Euxerus erythropus

N *Physaloptera abbreviata* Rudolphi, 1819
N *Syphacia obvelata* (Rudolphi, 1802) Seurat, 1916

Lemniscomys sp.

C *Hymenolepis diminuta* (Rudolphi, 1819) Weinland, 1858
N Trichostrongylinae (females only)

Taterillus sp.

N *Syphacia obvelata* (Rudolphi, 1802) Seurat, 1916

(c) Carnivora

Lutra maculicollis nilotica

T *Baschkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944

(d) Artiodactyla

Adenota kob leucotis

T *Schistosoma bovis* (Sonsino, 1876) Blanchard, 1895
N *Bunostomum phlebotomum* (Railliet, 1900)
N *Setaria cervi* (Rudolphi, 1819) Baylis, 1936

Bos taurus

N *Oesophagostomum radiatum* (Rudolphi, 1803)

Cephalophus sp.

C *Avitellina centripunctata* (Rivolta, 1874) Gough, 1911
N *Setaria cervi* (Rudolphi, 1819) Baylis, 1936

Damaliscus korrigan tiang

T *Fasciola gigantica* Cobbold, 1856

Kobus defassa

T *Paramphistomum cervi* (Schrank, 1790) Fischoeder, 1901
C *Stilesia hepatica* Wolffhügel, 1903
N *Bunostomum cobi* Maplestone, 1931
N *Setaria cervi* (Rudolphi, 1819) Baylis, 1936

Phacochoerus aethiopicus
N *Necator* sp. (females only)
N *Murshidia hamata* Daubney, 1923
Syncerus caffer aequinoctialis
T *Gastrothylax gregarius* (Looss, 1896) Stiles and Goldberger, 1910
T *Fasciola gigantica* Cobbold, 1856
N *Setaria cervi* (Rudolphi, 1819) Baylis 1936
Taurotragus sp.
N *Artionema hornbyi* (Boulenger, 1921) Liang-Sheng, 1959

In BIRDS
Ephippiorhynchus senegalensis
N *Paronchocerca ciconiarum* Peters, 1936
Milvus migrans parasiticus
C *Idiogenes travassosi* Ortlepp, 1938
T *Diplostomum tregenna* Gohar, 1932
T *Echinostoma* sp.
T *Opisthorchis geminus* (Looss, 1896) Looss, 1899
N *Habronema leptoptera* (Rudolphi, 1819) Seurat, 1914
N *Porrocaecum depressum* (Zeder, 1800) Baylis, 1920
Numida meleagris major
C *Hispaniolepis falsata* (Meggitt, 1927) Lopez-Neyra, 1942
C *Porogynia paronai* (Moniez, 1892) Railliet and Henry, 1909
N *Ascaridia numidae* (Leiper, 1908) Travassos, 1913
Pelecanus rufescens
T *Clinostomum complanatum* (Rudolphi, 1809) Braun, 1900
N *Contracaecum spiculigerum* (Rudolphi, 1809) Railliet and Henry, 1912
Phalacrocorax a. africanus
C *Idiogenes travassosi* Ortlepp, 1938
N *Contracaecum spiculigerum* (Rudolphi, 1809) Railliet and Henry, 1912
Ptilostomus afer
T *Athesmia heterolecithodes* Braun, 1899

In REPTILES
Agama mossambica
N *Strongyluris* sp. (females only)
N *Thelandros micruris* Rauther, 1918
Hemidactylus brooki angulatus
N *Pharyngodon* sp. (females only)
Mabuya quinquetaeniata
N *Thelandros micruris* Rauther, 1918
Naja haje
N *Kalicephalus obliquus* (Daubney, 1923) Ortlepp, 1923
Psammophis s. sibilans
N *Kalicephalus simus* (Daubney, 1923) Ortlepp, 1923

Varanus niloticus

C *Duthiersia fimbriata* (Diesing, 1850) Monticelli and Crety, 1891

T *Cyclorchis varani* Price, 1936

N Filarioidea sp. (fragments only)

In FISH

Lates niloticus

N *Contracaecum* type larvae

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NOTES

A HYMENOPTEROUS PARASITE OF SITONA SCISSIFRONS SAY (COLEOPTERA:CURCULIONIDAE)

CONRAD LOAN

Hymenopterous parasites of the adult of *Sitona* species have not hitherto been recorded from North America, but were reared in Europe by Jackson (3, 4, 5), Grossheim (2), Ulashkevitch (6), and Berry and Parker (1).

On May 28, 1959, I swept a number of *Sitona scissifrons* Say from a vetch, *Vicia angustifolia* Reichard var. *segetalis* (Thuill.) W. D. J. Koch, 2 miles west of the city of Belleville, Ontario. The vetch occurred in thick, discrete stands along the right-of-way of the Canadian National Railways. Forty-two per cent of 100 of these weevils dissected on June 8 contained parasite larvae. Final instar hymenopterous larvae emerged from the adult weevils from June 8 to June 25. Parasites were not found in or reared from either *S. cylindricollis* Fahr. or *S. hispidula* (F.) collected in the same locality.

Adults of this parasite emerged June 25 and specimens of both sexes were identified by Dr. W. R. M. Mason, Entomology Research Institute, Ottawa, Canada, as of an undescribed species of *Microctonus* Wesm. (Braconidae). A description of the species will be published by Dr. Mason.

It is planned to investigate the bionomics of the species and its importance as a mortality factor to *S. scissifrons* and related species.

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